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LC-HRMS AND NMR STUDY OF THE ESTERIFICATION PRODUCTS OF IBUPROFEN WITH SOLKETAL: FORMATION, ISOLATION, AND IDENTIFICATION

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Ibuprofen is a widely used non-steroidal anti-inflammatory drug dispensed in tablets, capsules, suspensions, oral solutions, creams, and gels. Ibuprofen's poor water solubility and gastrointestinal side-effects present ongoing formulation challenges. Alcoholic excipients are often employed to enhance solubility and minimise adverse effects. Solketal (1,2-isopropylidene glycerol), a ketal produced by the condensation of glycerol with acetone, offers further versatility as an excipient due to its free hydroxyl group, which enables esterification reactions with acidic active pharmaceutical ingredients like ibuprofen. Introducing any excipient, especially in direct contact with the active pharmaceutical ingredient, necessitates careful evaluation of potential drug–excipient interactions, as these can alter the drug's physicochemical properties and impact clinical performance. Chromatographic techniques coupled with mass spectrometry and nuclear magnetic resonance spectroscopy remain essential for identifying and characterizing related and degradation products in pre-formulation studies. In this study, we investigated the esterification of ibuprofen with solketal to identify possible interaction products. Two major compounds were isolated and thoroughly characterised by MS and NMR, confirming their chemical structures: 1-mono-glycerol ester of ibuprofen and ibuprofen-solketal-ester, which contained a 1,3-dioxolane ring. This finding highlights the importance of comprehensive analytical evaluation of drug–excipient interactions during formulation development, as these can affect drug stability and performance.

Keywords: ibuprofen; esterification; solketal; LC-HRMS; NMR

LC-HRMS И NMR СТУДИЈА НА ПРОИЗВОДИТЕ НА ЕСТЕРИФИКАЦИЈА НА ИБУПРОФЕН СО СОЛКЕТАЛ: ОБРАЗУВАЊЕ, ИЗОЛАЦИЈА И ИДЕНТИФИКАЦИЈА

Ибупрофенот е широко распростанет нестероиден антиинфламаторен лек кој постои во форма на таблети, капсули, орални суспензии, креми и гелови. Слабата растворливост на ибупрофенот во вода и несаканите гастроинтестинални ефекти претставуваат постојани предизвици за формулаторите на лекови. Алкохолни ексципиенти често се користат за подобрување на растворливоста и минимизирање на несаканите ефекти. Солкетал (1,2-изопропилиден глицерол), кетал произведен со кондензација на глицерол со ацетон, е разнообразен како ексципиент поради неговата слободна хидроксилна група, која овозможува реакции на естерификација со кисели активни фармацевтски состојки како што е ибупрофенот. Воведувањето на кој било ексципиент, особено оние во директен контакт со активната фармацевтска состојка, бара внимателна евалуација на потенцијалните интеракции помеѓу активната состојка и ексципиентот, бидејќи тие можат да ги променат физичко-хемиските својства на лекот и да влијаат на клиничките перформанси. Хроматографските техники во комбинација со масена спектрометрија и нуклеарна магнетна резонанца се неопходни за идентификација и карактеризација на сродни и деградациони продукти при предформулациските студии. Во рамките

на оваа студија беше испитана естерификацијата на ибупрофен со солкетал со цел идентификација на можни интеракциски производи. Два главни продукта беа изолирани и темелно карактеризирани со масена спектрометрија и нуклеарна магнетна резонанца, потврдувајќи ги нивните хемиски структури: 1-моно-глицерол естер на ибупрофен и ибупрофен-солкетал-естер со 1,3-диоксолански прстен. Ова откритие ја истакнува важноста на сеопфатната аналитичка евалуација на интеракциите помеѓу активната фармацевтска состојка и ексципиентите за време на развојот на формулацијата на фармацевтскиот производ, бидејќи тие можат да влијаат на стабилноста и перформансите на лекот.

Клучни зборови: ибупрофен; естерификација; солкетал; LC-HRMS; NMR

1. INTRODUCTION

The group of non-steroidal anti-inflammatory drugs (NSAIDs) comprises various drugs with proven non-narcotic analgesic, anti-inflammatory, and antipyretic properties. NSAIDs are considered among the most widely used drugs worldwide, due to their effectiveness in the treatment of symptoms such as pain and inflammation caused by conditions such as fever, rheumatoid arthritis, and ankylosing spondylitis.[1–3]

Ibuprofen (2-(4-isobutyilphenyl)propionic acid) is a well-known, commonly prescribed NSAID that acts as a prostaglandin inhibitor and belongs to the group of profens (2-arylpropionic acids).[1–7] Its structure is shown in Figure 1a. Due to the presence of the aromatic ring with alkyl substituents and a carboxylic group in the structure, the solubility of ibuprofen in aqueous acidic media in the stomach is limited.[3,5] On the other hand, long-term oral administration has been associated with different gastrointestinal side-effects, as it blocks the protection of the gastric mucosa.[4] This active pharmaceutical ingredient (API) is mainly administered in tablet form or suspensions and oral solutions, but there have been attempts to design formulations of ibuprofen that achieve therapeutic benefits despite its poor aqueous solubility and overcome the side-effects of oral use. Examples include: salt conjugates (lysinate, arginate); soft gelatin capsules filled with solubilised ibuprofen; and topical dermal dosage forms, commonly prepared from water with alcohol and a gelling agent.[3,5,7,8]

However, the introduction of any component in the formulation of the drug product, which comes into direct contact with the API, may lead to a reaction between the API and such component. These reactions (referred to as drug-excipient interactions) are carefully studied and their effects on the physicochemical properties of the drug product are closely monitored. In physical interactions, there is an alteration in physicochemical properties such as solubility, dissolution rate, organoleptic properties, polymorphic form, or crystallisation of the API, but no chemical reaction occurs. A chemical interaction involves a direct chemical reaction between the API and the excipient (or present impurities) and may result in the formation of new products, degradation, or loss of potency. It is important to note that such interactions may arise not only from the components themselves but also from their concentrations and the conditions present. While these interactions are possible, they can be controlled if identified early during drug product development. Moreover, in some cases, interactions between the drug and the excipient are intentionally designed to enhance the properties of the drug product, such as improving the solubility and bioavailability of the API.[7–13]

The identification of these reactions in the early development and prediction of the drug product stability can guide the selection of appropriate formulation components, the production process conditions, as well as support regulatory findings to justify proposed shelf life. The rate and extent of these reactions are influenced by factors such as chemical reactivity and ionisation of the compound, solvent content, presence of water, and manufacturing and storage conditions. Thus, if the possible reactions are known, the rate and extent can be limited by the formulator. An example is the formulation of soft gelatin capsules of ibuprofen where, during pre-formulation, several interaction products were identified along with possible strategies to prevent their formation in the final product.[7,11,14]

Consequently, specially designed compatibility studies are conducted during the pre-formulation phase and any detected reaction is recorded and analysed. For this purpose, binary mixtures of the API and the excipient are prepared in a ratio of 1:1 *(w/w)* – a worst-case scenario that maximises the contact amongst reactants – and subjected to elevated thermal and humidity techniques (usually at least 1 month). Samples are then analysed using different analytical techniques. Thermoanalytical techniques are mainly used for the detection of physical interactions. Chromatographic

techniques such as reversed-phase high-performance liquid chromatography (RP-HPLC) with various detectors are employed for studying chemical interactions. Characterisation of detected products with mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy has been established as an essential tool in early drug product development, contributing to awareness and early prediction of possible chemical interaction products,[12–15] high-resolution mass spectrometry (HRMS) being even more effective in providing data.

Studies have previously been performed in our laboratory to predict possible solid-state interactions of ibuprofen with different solid-state excipients. An interaction between ibuprofen and sodium hydrogen carbonate, as well as ibuprofen with magnesium stearate, was reported, providing experimental evidence of the formation of new molecular entities.[9,10] Regarding chemical interactions, ibuprofen–polyethylene glycol monoester and diester were reported in literature as products formed by the esterification of ibuprofen with polyethylene glycol, as well as ibuprofen–sorbitol ester and ibuprofen–sorbitan ester from the reaction with sorbitol and sorbitan. The use of these alcoholic excipients is well established as solvents in ibuprofen formulations for topical applications and as components of soft gelatin capsules.[7–11]

Glycerol (propane-1,2,3-triol, Fig. 1b), a non-toxic and non-irritating polyalcohol, is one of the most commonly used excipient in cosmetic, pharmaceutical, chemical, and food industries. The reaction between glycerol and ibuprofen is described in the literature, since the products of this reaction (mono-ester and di-ester between ibuprofen and glycerol) are often used in drug design for improved ibuprofen aqueous solubility.[4–6] On the other hand, due to the relatively easy synthesis, much attention has been dedicated to (2,2-dimethyl-1,3-dioxolane-4yl)methanol or solketal, hereafter. Solketal, also known as 1,2-isopropylidene glycerol, is a cyclic acetal (ketal) synthesised by condensation of glycerol with acetone, resulting in two possible solketal isomers: the isomer with a 1,3-dioxolane (five-membered ring, Fig. 1c) and the other isomer (2,2-dimethyl-1,3-dioxane-5-yl)methanol with a 1,3-dioxane (six-membered ring, Fig. 1d). However, the more desirable product for industry is the five-membered ring isomer, and when glycerol is converted to solketal, the five-membered ring is the major product.[16–19] Solketal, like glycerol is currently used as a solvent for pharmaceutical formulations. Due to the presence of a free hydroxyl group, solketal, like glycerol, remains available for esterification reactions.[16] Furthermore, since the reaction of glycerol with acetone is reversible in the presence of weak acids, the likelihood remains of obtaining different esterification products in the presence of a carboxylic acid such as ibuprofen.[16,18,19]

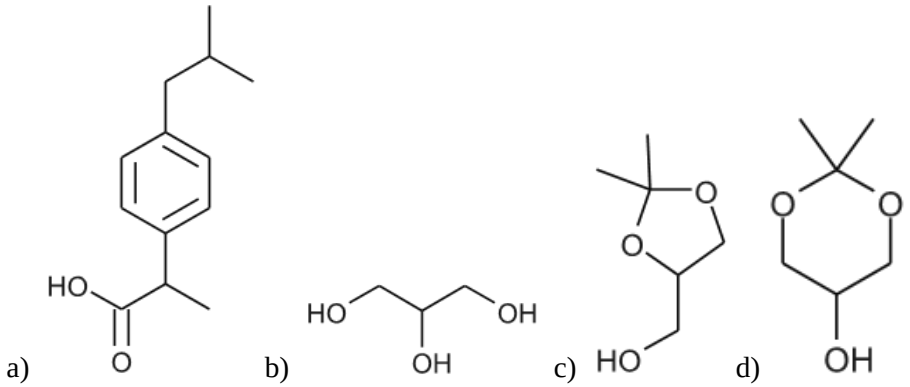


Fig. 1. Molecular structures of the reacting compounds: **a.** ibuprofen; **b.** glycerol; **c.** five-membered ring solketal isomer; **d.** six-membered ring solketal isomer

The aim of the present paper is to investigate the possibility of direct esterification of ibuprofen with solketal, in order to identify possible new products of a chemical interaction. An additional question is whether the same interaction products can be formed in the reaction between ibuprofen and solketal, as that obtained with glycerol. We suggest the possibility that, in presence of an acidic compound such as ibuprofen, solketal may undergo a ring-opening reaction to glycerol and result in the generation of the same product of chemical reaction as glycerol. To the best of the authors' knowledge, the drug–excipient interaction between ibuprofen and solketal has not yet been investigated.

The only work where a product from the esterification of ibuprofen with solketal was men-

tioned is a study on nanocarriers for the targeted delivery of NSAIDs for tumour therapy, where an ester between ibuprofen and solketal was briefly noted as an intermediate product in the process of obtaining specific phosphoester polymers.[20] Several different methods for synthesis, isolation, and purification of other ibuprofen esters have been described in the literature.[21,22] However, the available published literature data lack characterisation of the obtained products using MS and NMR spectroscopy.[21,22]

The products in the current work were synthesised by means of direct, acid catalysed, esterification, separated by RP-HPLC, purified using column chromatography on silica, and systematically characterised by thin-layer chromatography (TLC), MS, and NMR.

2. EXPERIMENTAL SECTION

2.1. *Chemicals, solvents, and standards*

Ibuprofen powder grade 38 was supplied as a non-commercial sample for research purposes, while solketal (1,2-isopropylidene glycerol) and glycerol (propane-1,2,3-triol, 85 %) were obtained as pharmaceutical-grade excipients provided as gift samples. Sulfuric acid (95 – 97 %) was manufactured by Alkaloid AD (Skopje, N. Macedonia), whereas formic acid, sodium carbonate, anhydrous sodium sulfate, *n*-hexane, ethyl acetate, acetonitrile, and silica gel for column chromatography 60 were sourced as analytical-grade reagents from Merck KGaA (Darmstadt, Germany). The reference standard 2,3-dihydroxypropyl 2-(4-isobutylphenyl)propanoate was purchased from Toronto Research Chemicals – TRC (Toronto, Canada).

2.2. *Procedure for esterification of ibuprofen*

For accelerated thermal degradation, binary mixtures in a ratio of 1:1 (*w*/*w*) of ibuprofen with solketal and ibuprofen with glycerol (85 %) were placed in a drying oven at a temperature of 80 °C for 66 and 42 h, respectively.

For isolation of the detected products, 50 g of ibuprofen powder was mixed with 90 ml of solketal. A catalytic amount (approximately 3 drops) of concentrated sulfuric acid was added, and the obtained solution was maintained under reflux at 100 °C, for 3.5 hours on a magnetic stirrer. After that, the solution was cooled to room temperature and repeatedly washed in a separatory funnel with a 1% solution of sodium carbonate to dissolve and remove the majority of unreacted ibuprofen. The extract was then washed repeatedly with water to remove any remaining solketal and sodium carbonate, then dried by shaking with anhydrous sodium sulfate. The obtained extraction mixture was stored over additional anhydrous sodium sulfate and evaluated using a previously optimised HPLC-DAD method to monitor the esterification products.

2.3. *HPLC method for evaluation of the extraction mixture*

All of the RP-HPLC-MS investigations were performed on a Vanqish UHPLC system coupled with a diode-array detector (DAD) and Orbitrap Exploris 120 High Resolution Mass Spectrometer (LC-HRMS) (Thermo Scientific Co., US). Chromatographic separations were conducted on a Zorbax Extend-C18 column (150 mm × 4.6 mm, 5 μm; Agilent Technologies, Inc., US). A mixture of 3 volumes of formic acid and 340 volumes of acetonitrile, diluted to 1000 volumes with water, was used as mobile phase A. Acetonitrile served as mobile phase B, and the following gradient was employed at a flow of 1.0 ml/min: 0 – 25 min 100 % A (v/v); 55 – 75 min 15 % A; and 72 – 80 min back to 100 % A.

The injection volume was 20 μl and the analysis was monitored at a wavelength of 265 nm. The mobile phases were used as solvents for all analysed samples. The instrument was controlled by Chromeleon and Xcalibur data acquisition and analysis software.

2.4. *Procedure for isolation and purification of the obtained products*

Column chromatography was carried out using approximately 17 g of silica gel 60 for column chromatography that had been conditioned with *n*-hexane. Different ratios of *n*-hexane and ethyl acetate were employed as mobile phases with increasing content of ethyl acetate. Fractions of 5 ml were collected throughout the separation process, and a previously optimised TLC method was applied for the detection of the products and for monitoring the separation process.

The first ten fractions (labelled 1–10) were eluted using 25 % ethyl acetate in *n*-hexane. Fractions 11–20 were collected with equal volumes of ethyl acetate and *n*-hexane, followed by elution with 80 % ethyl acetate for the final twenty fractions (labelled 21–40).

2.5. *TLC method*

For the TLC characterisation, silica gel 60 plates impregnated with fluorescent indicator F_{254} (Supelco®, Merck Analytical Products, Darmstadt, Germany) were used as the stationary phase, and a mixture of equal volumes of *n*-hexane and ethyl acetate served as the mobile phase. An aliquot of 20 µl from each fraction was applied onto the adsorbent. The visualisation of the plates was performed by irradiation at 254 nm in a UV UV cabinet 4 (CAMAG Co. Switzerland).

All fractions in which the TLC characterisation indicated the presence of a spot with the same characteristics (position, shape, size) were combined and concentrated under a nitrogen stream by evaporating the remaining solvent from the mobile phases.

2.6. *APCI-HRMS conditions*

For MS characterisation of the products, the following previously optimised conditions were applied: atmospheric pressure chemical ionisation (APCI) in positive ionisation mode was used, with the vaporising temperature set to 400 °C and the capillary temperature set to 300 °C. Sheath and auxiliary gas flow rates were set at 50 and 5 arbitrary units, with the sweep gas flow rate also set to 5 arbitrary units. Positive discharge current was set to 4 µA. MS spectra were acquired over a full acquisition range covering *m/z* 100–1000 and MS^2, fragmentation spectra for the most abundant fragments were obtained as well. The instrument was controlled by Chromeleon and Xcalibur data acquisition and analysis software and Mass Frontier spectral interpretation software.

2.7. *NMR conditions*

Proton nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a Bruker AVANCE II 400 NMR spectrometer operating at 400 MHz (100 MHz for ^{13}C NMR).

3. RESULTS AND DISCUSSION

3.1. *HPLC-UV-DAD results and characterisation of the products*

Chromatographic results using the RP-HPLC method with DAD as described in Section 2.3, after the accelerated thermal treatment of the binary mixtures, revealed the detection of four additional peaks in the ibuprofen/solketal binary mixtures, three of which were the same as those detected in the ibuprofen/glycerol binary mixture, indicating the possible generation of four new compounds. Due to the complexity of their collected MS spectra, the extraction process was conducted immediately and in parallel in order to obtain clearer spectra for characterisation.

The procedure for esterification of ibuprofen resulted in an extract that was prepared and purified as explained in Section 2.2, and it was first characterised using the same RP-HPLC method with UV-DAD. A chromatogram obtained from the resulting reaction mixture is shown in Figure 2, alongside the chromatograms from the treated binary mixtures. Collected UV spectra from the peak of each reported compound in the chromatogram are provided in Supplementary Material, Figure S1. Standards of ibuprofen were used for identification of ibuprofen in the reaction mixture. Any peak in the chromatogram previously assigned to known ibuprofen-related and degradation products in small amounts and/or solvents and mobile phases were not considered.

Chromatographic results after the extraction process indicated that only ~3.44 % of ibuprofen remained unreacted in the mixture. Regarding the presence and abundance of other constituents of the obtained mixture, two major products were evident at retention times ~26.55 min (content 51.00 %) and ~47.49 min (content 36.64 %), respectively. The relative retention times (RRT = $T_{analyte}/T_{reference}$), calculated with reference to the retention of ibuprofen (~36.11 min), were 0.74 for the early-eluting major product marked as P1, and 1.32 for the later-eluting major product marked P2. These two products were subsequently isolated, purified using column chromatography, and identified.

Two other minor products were detected in the chromatogram of the extraction mixture with RRTs of 0.66 and of 1.53 (contents of 3.49 % and 2.42 %, respectively). However, the contents of these products were considered too low for isolation, so only their UV and mass spectra were studied for identification.

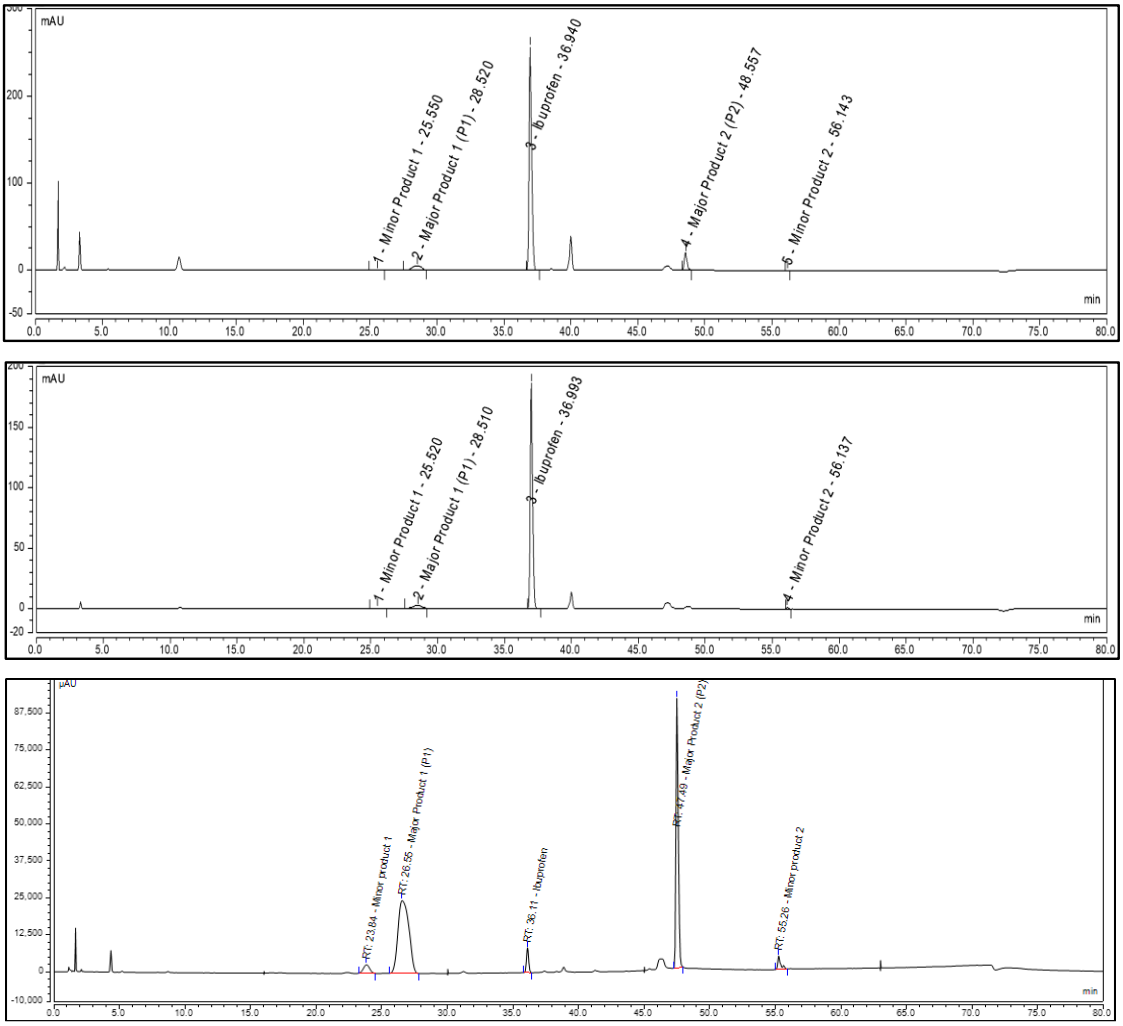


Fig. 2. HPLC results with marked peaks of ibuprofen and detected products. **Top:** Chromatogram at 265 nm of ibuprofen/solketal binary mixture; **Middle:** Chromatogram at 265 nm of ibuprofen/glycerol binary mixture; **Bottom:** Chromatogram at 265 nm of the mixture after the extraction process. Additional peaks previously assigned to the mobile phase, solvent, and known ibuprofen-related and degradation products in small amounts were not considered.

3.2. *TLC results and characterisation of the products*

The progress of the esterification process was monitored using TLC as described in Section 2.5. The appearance of two major spots on the TLC plate of the reaction mixture, eluting before and after ibuprofen, was observed simultaneously with the decrease of the ibuprofen spot. This observation was consistent with the HPLC results. The two minor products detected in the HPLC chromatograms were not visible in TLC due to their low abundance. The resulting TLC plates are shown in Supplementary Material, Figure S2.

The excellent TLC separation on silica indicated that column chromatography on silica would be suitable for isolation the major products P1 and P2 and that the process could be monitored by TLC. The resulting TLC plates from the fractions labelled 6–10 are shown in Figure S2b (first-eluting compound on silica, P2, which corresponds to the later-eluting major product on reversed-phase HPLC). Later-eluting fractions numbered 29–35 (shown in Fig. S2c) were combined and attributed to the major product P1 (in the RP-HPLC chromatogram), which was well separated from ibuprofen and P2.

The TLC results for the two isolated products corresponded to the results obtained for the extraction mixture, demonstrating that the major products P1 and P2 had been isolated in good yield and purity.

3.3. *Characterisation by HPLC-APCI-HRMS*

The peak of ibuprofen in the extraction mixture was identified not only by retention time (t_R) and comparison with the standard but also by the obtained mass spectra. The full MS spectrum in positive ionisation mode collected for the ibuprofen peak at t_R ~ 36 min (Fig. 3) revealed a small peak at *m/z* 207.14 attributed to a protonated molecular ion $[M+H]^+$ of

ibuprofen (M_r = 206.3).[23] A peak at *m/z* 224 was also present in the spectrum, corresponding to a molecular ion of ibuprofen with an adduct of +18 amu. Such adducts are usually attributed to ammonia adducts, but since no ammonia was present in the source, it was likely due to residual ammonia in the system, often used as a mobile phase constituent and ionisation-enhancing additive. Adducts were expected to form since adduct formation is one of the main mechanisms of APCI ionisation.[24–26]

The possibility of ammonia adduct formation during ionisation was checked and confirmed by adding ammonium formate addition to the mobile phase, as cluster ion formation is a well-described phenomenon when using APCI ionisation.[27,28] A very intensive signal at *m/z* 161 was due to the ion generated after a loss of 45 amu, which was observed in all ibuprofen and ibuprofen-related spectra.[11] This fragment was attributed to a product of decarboxylation, i.e., cleavage of the carboxylic group from the structure of ibuprofen. The signal at *m/z* 161.13 was also the base peak present in the MS^2 spectra considered (Fig. 3).

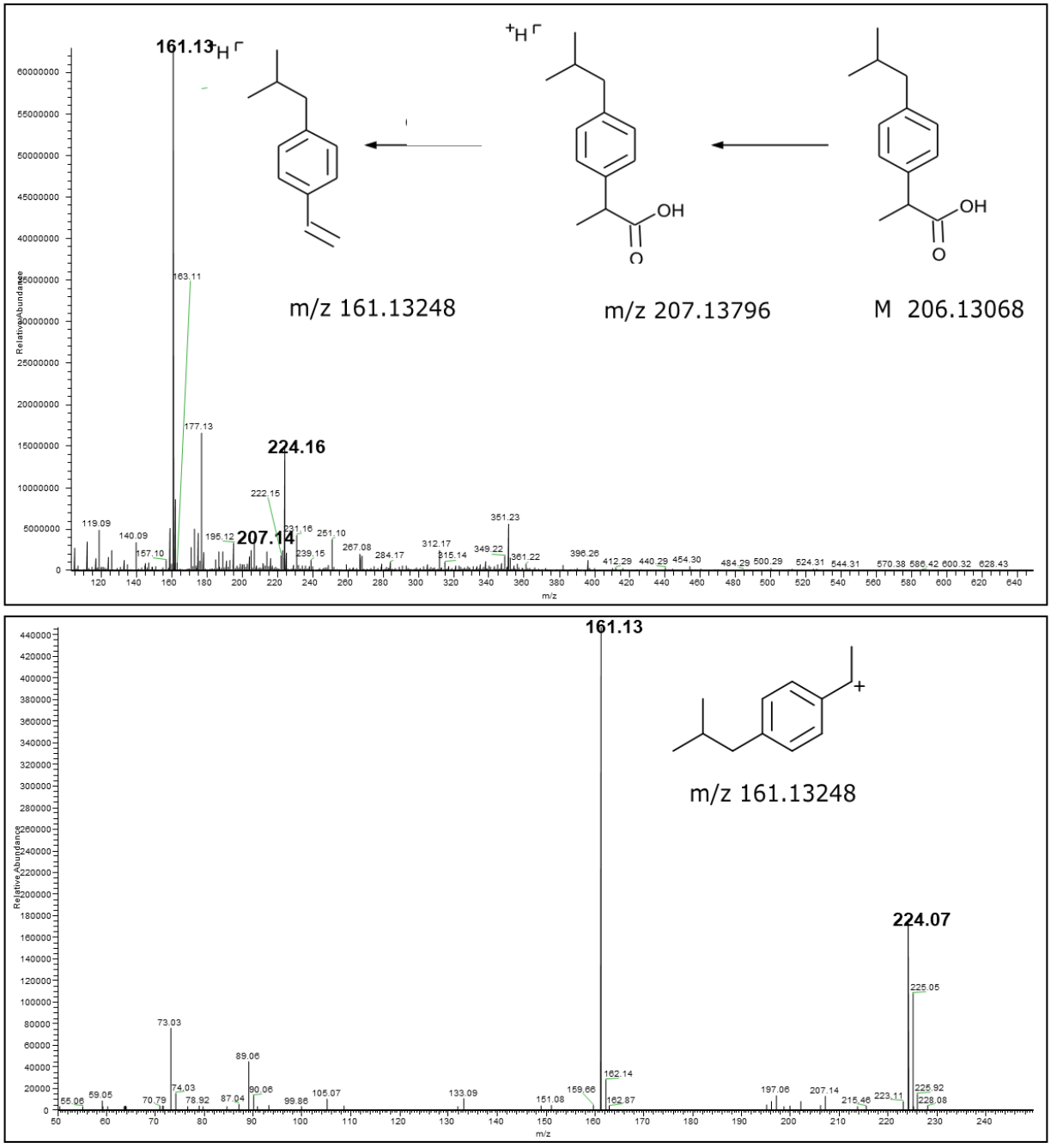


Fig. 3. Mass spectra collected from the peak of ibuprofen. **Top**: full MS spectrum; **Bottom**: MS^2 spectrum from the molecular ion [M+18 amu adduct]

MS spectra collected from the analysis of the product labelled as P1 are shown in Figure 4. The full MS spectrum revealed a peak from the protonated molecule at *m/z* 281.17, accompanied by a peak at *m/z* 298.20 (an expected adduct of +18 amu), both corresponding to a relative molecular weight of 280. This proposed molecular weight matched the hypothesised structure of an ibuprofen–glycerol monoester (M_r = 280), also shown in Figure 4. The base peak at *m/z* 263.16 obtained during in-source fragmentation was attributed to the ion formed after a loss of 18 amu, corresponding to cleavage of a water molecule from the available hydroxyl groups. The fragment ion at *m/z* 161.13 was again present in the spectrum, as is typical for all ibuprofen-related compounds, indicating a structure related to ibuprofen.

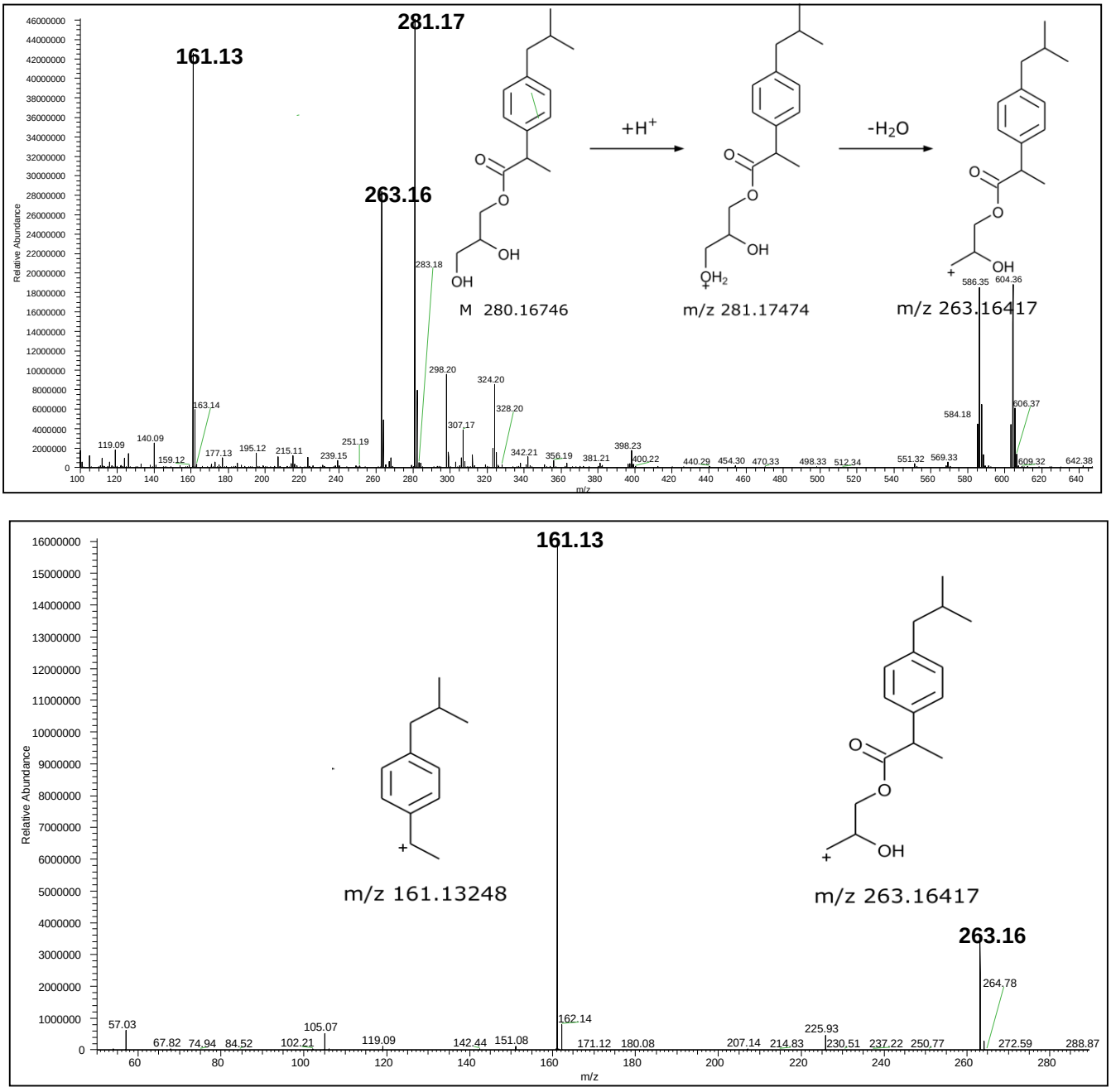


Fig. 4. Mass spectra collected for the peak of the major product P1.
Top: full MS spectrum; **Bottom**: MS^2 spectrum from the peak at *m/z* 263

Two additional peaks with *m/z* much larger than the proposed molecular weight were observed in the spectrum at *m/z* 586.35 and 604.36. These peaks were attributed to in-source noise from column residues and to the formation of water and ammonia cluster adducts. These fragments corresponded to large clusters formed from the molecular ions with the addition of 17 and 18 adducts of 18 amu units, respectively (280 + 306 = 586 and 280 + 324 = 604). Such adduct formation has been reported when analysing compounds such as esters and NSAID derivatives using APCI, due to their affinity to be ionized via adduct formation.[27,29,30]

In the MS^2 spectrum of the fragment at *m/z* 263.16 (Fig. 4), only the fragments at *m/z* 263.16 and *m/z* 161.13 were notable, which further supported this tentative structural elucidation.

An additional confirmation of the proposed structure of P1, since a reference standard of an ibuprofen–glycerol ester was commercially available, was obtained and analysed using the same HPLC-APCI-MS method and the TLC method. The obtained chromatograms are shown in Supplementary Material, Figure S3. A match in the retention time of P1 and the peak from the standard solution in both TLC (Fig. S3a) and HPLC (Fig. S3b), as well as the MS spectra (Fig. S3c), was considered confirmation of the structure of the major product P1.

Thus, the formation of ibuprofen–glycerol ester obtained by 1,3-dioxolane ring opening of solketal in the presence of ibuprofen was confirmed, and an ester between ibuprofen and glycerol was identified as a possible product, even though it was not present in the formulation. The collected MS spectra (Fig. S3c) from the obtained standard solution corresponded closely to the discussed MS spectra of P1. Furthermore, the fragments at higher *m/z* (586.35 and 604.36), assumed to result from in-source noise or adduct formation during ionisation, were also present in the mass spectra of the reference compound solution and therefore confirmed their origin.

As additional confirmation of the hypothesis that the same esterification reaction producing the glycerol ester of ibuprofen also occurred between ibuprofen and solketal, the chromatogram from the ibuprofen/glycerol binary mixture was examined (previously presented in Fig. 2). A peak at the same retention time as that of the ibuprofen-1-mono-glycerol ester was observed in both chromatograms.

At this point, the presence of the previously discussed minor product from the extraction mixture at RRT ~ 0.66 had been detected in all studied chromatograms: in the reference standard solution of 1-monoglycerol ester of ibuprofen, as well as in both prepared and treated binary mixtures. The MS spectra collected for this peak in the extraction mixture are presented in Figure 5. As shown, the collected MS spectra completely matched the MS spectra obtained for P1, with the same ions identified (at *m/z* 586.35, 281.17, 263.16, and 161.13). Such resemblance in the MS spectra indicated the formation of a possible isomer in a smaller quantity, which was likely ibuprofen-2-mono-glycerol ester.

The MS spectra collected during the analysis of the major product labelled as P2 are shown in Figure 6. The full MS spectrum in positive ionisation mode revealed a signal at *m/z* 321, corresponding to a protonated molecular ion $[M+H]^+$ of a compound with a relative molecular weight of 320. If a possible product formed via direct esterification between ibuprofen (M_r = 206.28) and solketal (M_r = 132.16) was considered, the relative molecular weight of this structure (M_r = 320, calculated as 206 + 132 – 18) supported the proposition that the ion at *m/z* 321 was the molecular peak. The base peak at *m/z* 263.16 was likely a result of 1,3-dioxolane ring opening caused by in-source fragmentation, and subsequent cleavage of an –O–$CH(CH_3)_2$ fragment (Fig. 6). This peak at *m/z* 263.16 coincided with the fragment obtained after a loss of a water molecule from the mono-glycerol ester of ibuprofen (P1) mentioned earlier. The fragment at *m/z* 161.13, characteristic of all ibuprofen-related compounds, was also the main peak observed in the MS^2 spectra of both ions under discussion (Fig. 6). Therefore, according to the MS results, the product labelled as P2 was characterised as an ibuprofen–solketal ester. The elucidated structure of ibuprofen–solketal ester is presented in Figure 6.

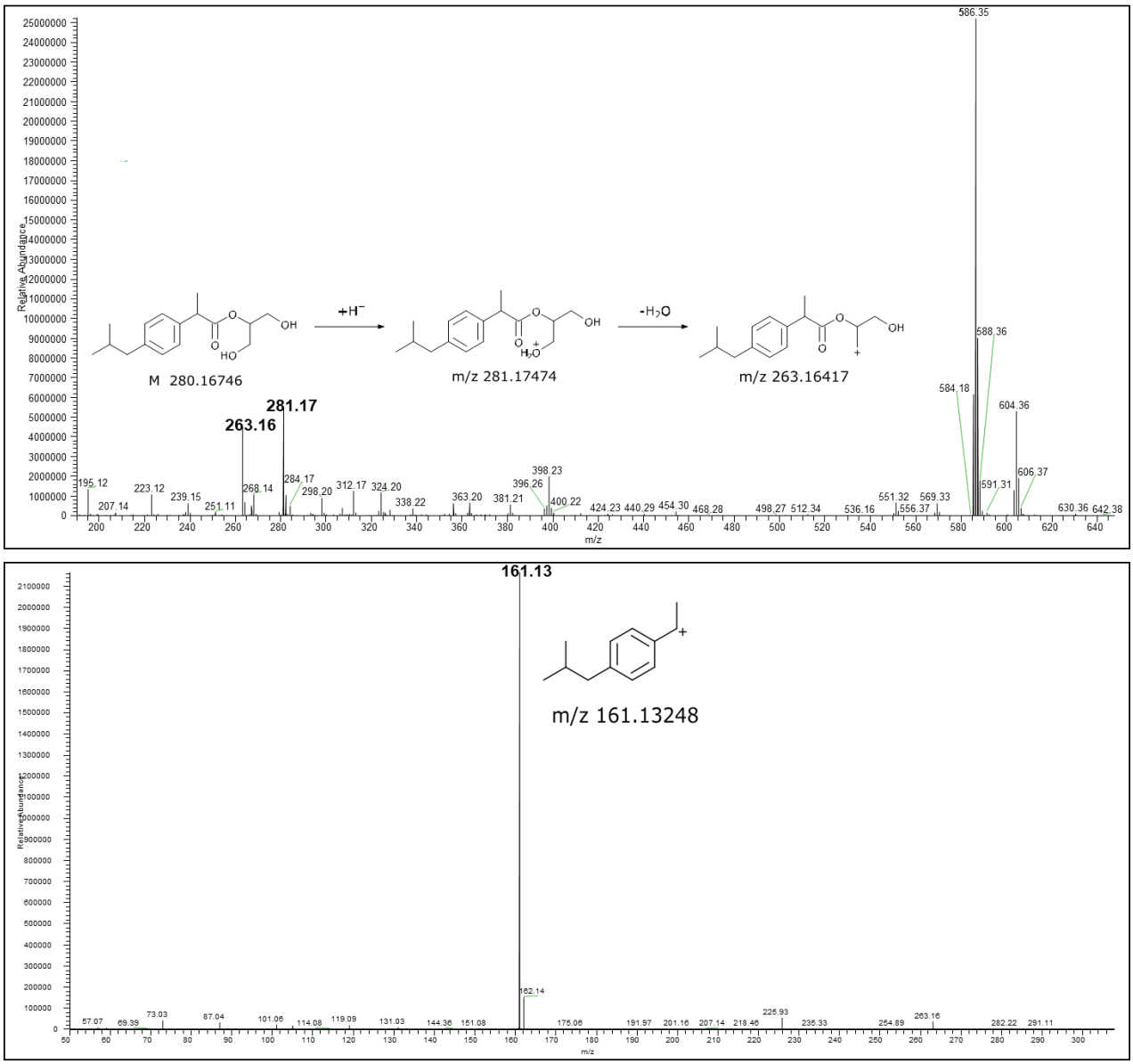


Fig. 5. Mass spectra collected from the minor product at RRT ~ 0.66. **Top**: full MS spectrum; **Bottom**: MS^2 spectrum from the peak at *m/z* 281

In the initial extraction mixture, a small amount of another minor compound (with RRT ~ 1.53) was detected, but it was sufficiently abundant for isolation and purification. The MS spectra obtained for this peak during the analysis of the extraction mixture are presented in Figure 7.

The main peaks in the full MS spectrum observed at *m/z* 451 and 486, combined with the higher retention values, suggested a more complex and less polar structure. If a di-ester of ibuprofen (M_r = 206) and glycerol (M_r = 92) was considered as a tentative structure (with $M_r = 2 \times 206 + 92 - 2 \times 18 = 468$), the peak at *m/z* 486 would have corresponded to an adduct of +18 amu to the molecular ion, which remained present throughout the analysis. Additionally, the fragment at *m/z* 451 corresponded to an ion obtained after an in-source fragmentation loss of 18 amu, a water molecule, due to a loss of the last available hydroxyl group.

In the obtained MS^2 spectrum (Fig. 7), the fragments at *m/z* 263.16 and 161.13 were the most significant. The fragment at *m/z* 263.16 resulted from the cleavage of one ibuprofen ester bonds (a loss of 205 amu) and is characteristic of a monoester of ibuprofen with glycerol. This fragment was followed by the typical ibuprofen fragmentation pattern, producing the fragment at *m/z* 161.13.

However, due to the small amount obtained in the mixture, this product could not be isolated; therefore, the structure of such ibuprofen-glycerol diester remained tentative, with the prospect of further experimentation. The elucidated structure of ibuprofen-glycerol diester is presented in Figure 7.

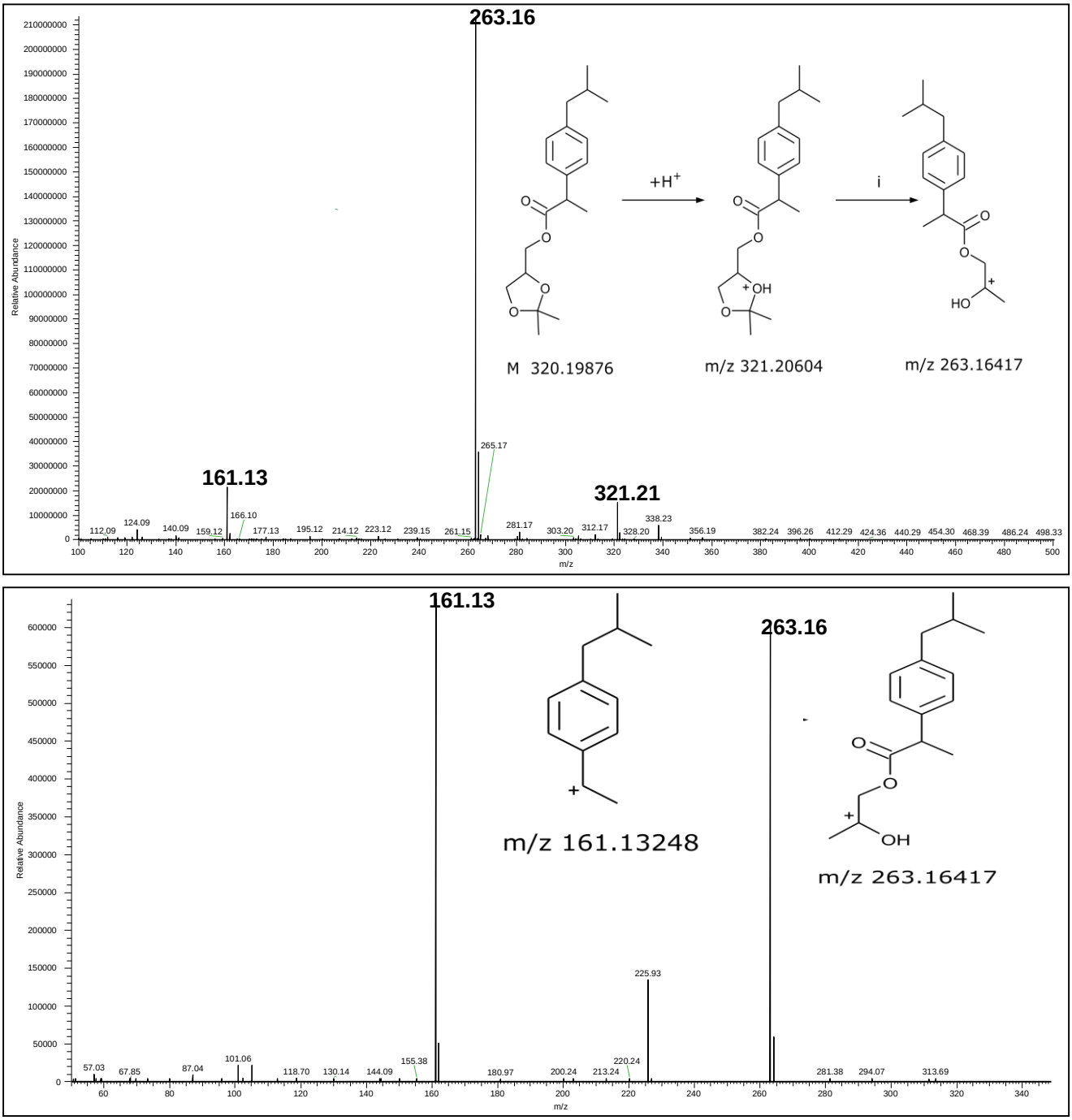


Fig. 6. Mass spectra collected from the peak P2. **Top**: full MS spectrum; **Bottom**: MS^2 spectrum from the peak at *m/z* 321

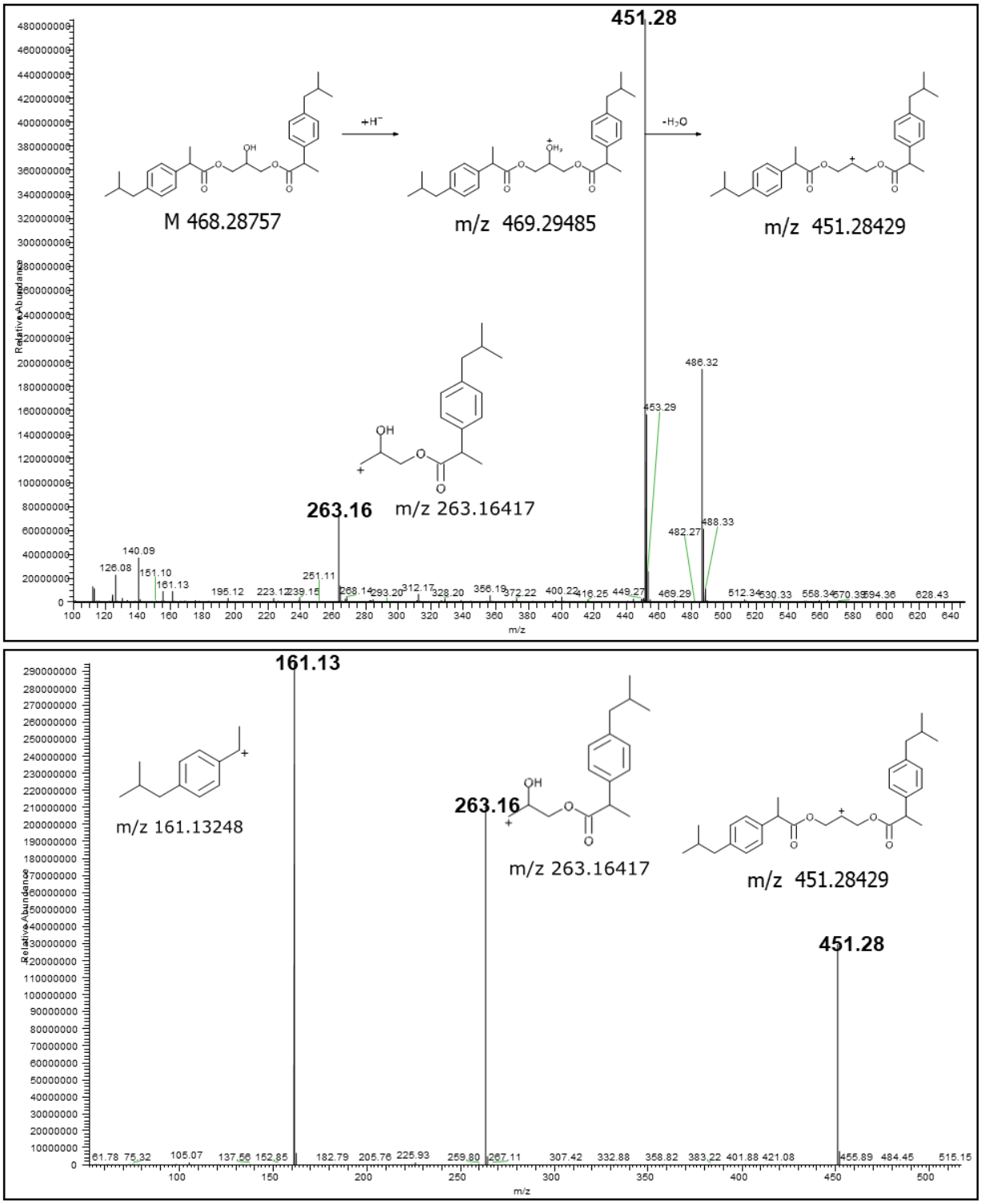


Fig. 7. Mass spectra collected from the product at RRT ~ 1.53. **Top**: full MS spectrum; **Bottom**: MS^2 spectrum from the peak at *m/z* 451

3.4. *Characterisation by ^1H and ^{13}C NMR*

To confirm the structures proposed from the study of the mass spectra of the major products, they were isolated and characterised by NMR (1D and 2D).

As shown in Table 1 and in Figure S4, the ^1H and ^{13}C NMR spectra of P1 corresponded to a structure representing an ester between ibuprofen and glycerol and matched the data published in previous studies on ibuprofen-1-mono-glycerol ester.[5,20] Thus, it was positively identified as an

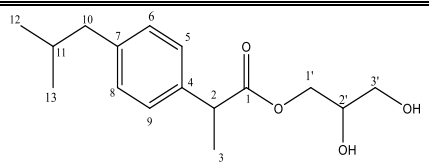
equimolar mixture of diastereoisomers (at C2') ibuprofen-1-glycerol ester.

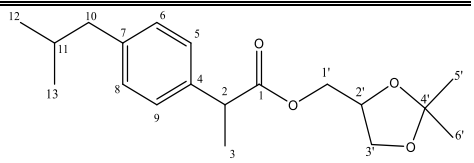
Compound P2 showed NMR spectra similar to P1 in the ibuprofen moiety; however, the difference lay in the alcohol moiety of the ester, which displayed the signals of solketal.[31] The spectra coincided with the ^{1}H and ^{13}C NMR data previously reported for the ibuprofen–solketal ester, containing a five-membered ring, obtained as an intermediate product in another study on phosphoester polymers for nanocarriers for cancer therapy.[20]

The structure was further supported through the Heteronuclear Multiple Bond Correlation (HMBC) of methylene protons of the CH_2-1' group (δ_H 4.12, m) to C-1 and methylene protons of CH_2-3' (δ_H 3.95 and 3.62, m) to C-1' and C-4'.

T a b l e 1

^{1}H and ^{13}C NMR data of P1 and P2 in $CDCl_3$ (^{1}H at 400 MHz, ^{13}C at 100 MHz, δ/ppm, J/Hz)





	P1 ($C_{16}H_{24}O_4$, M_r = 280)		**P2** ($C_{19}H_{28}O_4$, M_r = 320)	
	^{1}H	^{13}C **	^{1}H	^{13}C **
1	–	175.13/175.09	–	174.43/174.41
2	3.72 q (7.1Hz)	44.94	3.73 q (7.1Hz)	44.93/44.89
3	1.48 (7.1 Hz)	18.27/18.25	1.50 d (7.1 Hz)	18.35/18.32
4	–	137.31	–	137.39
5,9	7.18 d (8.0 Hz)	127.01	7.20 d (8.0 Hz)	127.06/127.00
6,8	7.09 d (8.0 Hz)	129.34	7.09 d (8.0 Hz)	129.36/129.26
7	–	140.67	–	140.54/140.52
10	2.44 d (7.2 Hz)	44.91	2.44 d (7.2 Hz)	44.89
11	1.84 m	30.07	1.84 m	30.08
12 and 13	0.89 d (6.7 Hz)	22.27	0.89 d (6.7 Hz)	22.27
1'	4.10 m	65.26/65.23	4.12 m	64.64/64.19
2'	3.81 brs	70.00/69.94	4.23 m	73.40/73.38
3'	3.53 m 3.44 m	63.14	3.95 m 3.62 m	66.20/66.09
4'			–	109.59/109.50
5'*			1.36 s	26.51/26.49
6'*			1.33 br s	25.34/25.29

*Interchangeable signals within columns; **Most of the signals are doubled due to two diastereoisomers (1:1)

4. CONCLUSION

Gathering information about the possible reaction between an API and an excipient and characterising the detected products with LC/MS is a necessary step in drug product development, as demonstrated in this study of ibuprofen and solketal. After the initial results from a binary mixture had indicated the possibility of four reaction products, direct esterification of ibuprofen with solketal, to characterise the possible chemical interaction products, was carried out using direct esterification. The products were isolated through HPLC, purified by column chromatography, and characterised by TLC, MS, and NMR.

Two major products were identified and characterised as follows: 1) ibuprofen-1-mono-glycerol ester and 2) an ibuprofen–solketal ester (with a 1,3-dioxolane ring). The esterification of ibuprofen with solketal had not previously been reported as an interaction and, to best of the authors' knowledge, the possibility of obtaining an ibuprofen–glycerol product via esterification of solketal with ibuprofen and 1,3-dioxolane ring opening in the presence of ibuprofen had not yet been reported.

Although these chemical interactions may not occur in the ratios present in the dosage form and under milder conditions, comprehensive knowledge of their potential formation was crucial for optimising drug product development. These findings provided added value by enabling early

detection and awareness of possible chemical interaction products between ibuprofen and solketal, as well as their potential intentional use.

Finally, further investigations could be undertaken to characterise the two minor possible products from the esterification of ibuprofen with solketal, tentatively identified as: ibuprofen-2-mono-glycerol ester and ibuprofen-glycerol diester.

REFERENCES

(1) Day, R. O.; Graham, G. G., Non-steroidal anti-inflammatory drugs (NSAIDs). *Brit. J. Sport. Med.* **2014**, *48* (18), 1396.2–1396. https://doi.org/10.1136/bmj.f3195

(2) Veloso, C.; Cardoso, C.; Vitorino, C., Topical fixed-dose combinations: A way of progress for pain management? *J. Pharm. Sci.* **2021**, *110* (10), 3345–3361. https://doi.org/10.1016/j.xphs.2021.06.009

(3) Irvine, J.; Afrose, A.; Islam, N., Formulation and delivery strategies of ibuprofen: challenges and opportunities. *Drug Dev. Ind. Pharm.* **2018**, *44* (2), 173–183. https://doi.org/10.1080/03639045.2017.1391838

(4) Toledo, M. V.; José, C.; Suster, C. R. L.; Collins, S. E.; Portela, R.; Bañares, M. A.; Briand, L. E., Catalytic and molecular insights of the esterification of ibuprofen and ketoprofen with glycerol. *Mol. Catal.* **2021**, *513*, 111811. https://doi.org/10.1016/j.mcat.2021.111811

(5) Zappaterra, F.; Presini, F.; Venturi, V.; Lerin, L. A.; Giovannini, P. P.; Costa, S., Biocatalytic insights for the synthesis of new potential prodrugs: Design of two ibuprofen derivatives. *Appl. Sci-Basel.* **2023**, *13* (17). https://doi.org/10.3390/app13179852

(6) Ravelo, M.; Fuente, E.; Blanco, Á.; Ladero, M.; García-Ochoa, F., Esterification of glycerol and ibuprofen in solventless media catalyzed by free CALB: Kinetic modelling. *Biochem. Eng. J.* **2015**, *101*, 228–236. https://doi.org/10.1016/j.bej.2015.06.002

(7) Douša, M.; Meca, L.; Gibala, P.; Jirman, J.; Tkadlecová, M.; Srbek, J.; Šalandová, J.; Kovalčíková, E.; Břicháč, J., Esterification of ibuprofen in soft gelatin capsules formulations – identification, synthesis and liquid chromatography separation of the degradation products. *J. Chromatogr. Sci.* **2017**, *55* (8), 790–797. https://doi.org/10.1093/chromsci/bmx036

(8) Gee, C. M.; Watkinson, A. C.; Nicolazzo, J. A.; Finnin, B. C., The effect of formulation excipients on the penetration and lateral diffusion of ibuprofen on and within the stratum corneum following topical application to humans. *J. Pharm. Sci.* **2014**, *103* (3), 909–919. https://doi.org/10.1002/jps.23850

(9) Bogatinovska, E. C.; Geškovski, N.; Petrushevski, G.; Stefov, V., Multivariate analysis for rapid screening and prediction of solid-state compatibility in pharmaceutical preformulation studies – Paving the road for machine learning. *Maced. J. Chem. Chem. Eng.* **2024**, *43* (1), 99–113. https://doi.org/10.20450/mjcce.2024.2838

(10) Stojanovska Pecova, M.; Geskovski, N.; Petrushevski, G.; Chachorovska, M.; Krsteska, L.; Ugarkovic, S.; Makreski, P., Solid-state interaction of ibuprofen with magnesium stearate and product characterization thereof. *Drug Dev. Ind. Pharm.* **2020**, 1308–1317. https://doi.org/10.1080/03639045.2020.1788067

(11) Farias, F. F., Martins, V. A. P.; Dörr F. A.; Trujillo, L. M.; Pinto E., Forced degradation study and characterization of main impurities of ibuprofen soft gelatin capsules by LC-MS-QTOF. *Pharmazie* **2021**, *76* (4), 138–144. https://doi.org/10.1691/ph.2021.0126

(12) Liltorp, K.; Larsen, T. G.; Willumsen, B.; Holm, R., Solid state compatibility studies with tablet excipients using nonthermal methods. *J. Pharm. Biomed. Anal.* **2011**, *55* (3), 424–428. https://doi.org/10.1016/j.jpba.2011.02.016

(13) Chadha, R.; Bhandari, S., Drug-excipient compatibility screening-Role of thermoanalytical and spectroscopic techniques. *J. Pharm. Biomed. Anal.* **2014**, *87*, 82–97. https://doi.org/10.1016/j.jpba.2013.06.016

(14) Gullapalli, R. P., Soft gelatin capsules (softgels). *J. Pharm. Sci.* **2010**, 99 (10), 4107–4148. https://doi.org/10.1002/jps.22151

(15) Pan, C.; Liu, F.; Motto, M., Identification of pharmaceutical impurities in formulated dosage forms. *J. Pharm. Sci.* **2011**, 100 (4), 1228–1259. https://doi.org/10.1002/jps.22376

(16) Samoilov, V. O.; Ni, D. S.; Maximov, A. L., Transacetalization of Solketal: A greener route to bioglycerol-based speciality chemicals. *Chemistry Select* **2018**, *3* (33). https://doi.org/10.1002/slct.201802135

(17) Moity, L.; Benazzouz, A.; Molinier, V.; Nardello-Rataj, V.; Elmkaddem, M. K.; de Caro, P.; Thiébaud-Roux, S.; Gerbaud, V.; Marion, P.; Aubry, J. M., Glycerol acetals and ketals as bio-based solvents: Positioning in Hansen and COSMO-RS spaces, volatility and stability towards hydrolysis and autoxidation. *Green Chem.* **2015**, *17* (3), 1779–1792. https://doi.org/10.1039/c4gc02377c

(18) Perosa, A.; Moraschini, A.; Selva, M.; Noè, M., Synthesis of the fatty esters of solketal and glycerol-formal: Biobased specialty chemicals. *Molecules* **2016**, *21* (2). https://doi.org/10.3390/molecules21020170

(19) Da Silva, M. J.; Rodrigues, A. A.; Pinheiro, P. F., Solketal synthesis from glycerol and acetone in the presence of metal salts: A Lewis or Brønsted acid catalyzed reaction. *Fuel* **2020**, *276*. https://doi.org/10.1016/j.fuel.2020.118164

(20) Akkad, M. S., *Nanocarriers for the Targeted Delivery of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) to Tumors*. PhD thesis, University of Kent, 2020. https://kar.kent.ac.uk/id/eprint/84710

(21) Shapiro, M., Characterization of unknowns in an NSAID gel formulation via oxymercuration. *Pharmaceuticals* **2009**, July/August, 10–12. Available at: https://www.researchgate.net/publication/325846956

(22) Abualhasan, M.; Assali, M.; Jaradat, N.; Tarayra, R.; Hamdan, A.; Ardah, R.; Zaid, A. N., Synthesis and formulation of Ibuprofen pro-drugs for enhanced transdermal absorption. *Int. J. Pharm. Pharm. Sci.* **2015**, *7* (2), 352–354. Available at:

https://journals.innovareacademics.in/index.php/ijpps/article/view/3650

(23) Ibuprofen, *Ph. Eur.* 11.6, 0721 (01/2017).

(24) Byrdwell, W. C., Atmospheric pressure chemical ionization mass spectrometry for analysis of lipids. *Lipids* **2001**, *36*, 327–346. https://doi.org/10.1007/s11745-001-0725-5

(25) Honing, M.; Van Bockxmeer, E.; Beekman, D., Adduct formation of steroids in APCI and its relation to structure identification. *Analusis* **2000**, *28* (10), 921–924. https://doi.org/10.1051/analusis:2000280921

(26) Terrier, P.; Desmazières, B.; Tortajada, J.; Buchmann, W., APCI/APPI for synthetic polymer analysis. *Mass Spectrom. Rev.* **2011**, *30* (5), 854–874. https://doi.org/10.1002/mas.20302

(27) Kostiainen, R.; Kauppila, T. J., Effect of eluent on the ionization process in liquid chromatography-mass spectrometry. *J. Chromatogr. A* **2009**, *1216* (4), 685–699. https://doi.org/10.1016/j.chroma.2008.08.095

(28) Klee, S.; Derpmann, V.; Wißdorf, W.; Klopotowski, S.; Kersten, H.; Brockmann, K. J.; Benter, T.; Albrecht, S.; Bruins, A. P.; Dousty, F.; Kauppila, T. J.; Kostiainen, R.; O'Brien, R.; Robb, D. B.; Syage, J. A., Are clusters important in understanding the mechanisms in atmospheric pressure ionization? Part 1: Reagent ion generation and chemical control of ion populations. *J. Am. Soc. Mass Spectrom.* **2014**, *25* (8), 1310–1321. https://doi.org/10.1007/s13361-014-0891-2

(29) Schmit, J. P.; Dawson, P. H.; Beaulieu, N., Chemical synthesis inside the collision cell of a MS/MS system: 1-formation of adduct ions between protonated esters and ammonia. *J. Mass Spectrom.* **1985**, *20* (4), 269–175. https://doi.org/10.1002/oms.1210200402

(30) Ohta, M.; Kawakami, N.; Yamato, S.; Shimada, K., Analysis of acetaminophen glucuronide conjugate accompanied by adduct ion production by liquid chromatography–atmospheric pressure chemical ionization-mass spectrometry. *J. Pharm. Biomed. Anal.* **2003**, *30* (6), 1759–1764. https://doi.org/10.1016/s0731-7085(02)00518-6

(31) Gilmutdinova, A. A.; Gubskaya, V. P.; Fazleeva, G. M.; Latypov, S. K.; Zhelonkina, T. A.; Sharafutdinova, D. R.; Nuretdinov, I. A.; Sinyashin, O. G., Synthesis and properties of new fullerene C60 derivatives, containing acetonide and polyol fragments. *Tetrahedron* **2014**, *70* (35), 5947–5953. https://doi.org/10.1016/j.tet.2014.06.009