

Application Note

High Sensitivity Metabolite Screening with ionKey/MS Using Post-Column Addition

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Abstract

This application note illustrates the enhanced sensitivity obtained by using post column addition of isopropanol by analysis of ibuprofen metabolites in urine as an example.

Benefits

The ionKey/MS System allows for high-sensitivity detection of polar metabolites with improved ionization process through post-column addition.

Introduction

The importance of metabolite profiling of a new drug is well-recognized. Elucidation of drug metabolite information is crucial due to the fact that drug metabolites can be toxic at certain levels, have a greater pharmacodynamic effect than the parent drug, interfere with concomitant medication, and impact liver function. In drug discovery, liquid chromatography (LC) combined with electrospray ionization (ESI) mass spectrometry (MS) has become the method of choice for obtaining rapid metabolic information due to its ease of use, exceptional selectivity, and applicability, even toward thermally labile polar metabolites, such as glucuronides, sulfates, and glutathione conjugates.

However, in LC/ESI-MS the analyte sensitivity is dependent upon the nature of the analyte, as well as the mobile-phase properties, such as organic solvent and electrolyte contents.

It is accepted that a mobile phase with a higher composition of organic solvents improves the ionization efficiency, especially in the case of negative polarities.¹ However, achieving the desired chromatographic separation of metabolites may require the use of mobile phases that may not be ideal for ESI. Altering solvent properties by post-column addition of a modifier can be an effective technique to improve sensitivity. Post-column addition of an organic modifier stabilizes the spray and allows enhancement of sensitivity without affecting the chromatographic separation.

An attractive aspect of the post-column addition is that the LC part is completely decoupled from the ionization region, which allows the use of an assortment of modifiers.

The post-column addition iKey (PCA iKey, Figure 1) used here is a microfluidic device containing two

channels, one channel (the separation channel) is packed with sub-2- μm reversed-phase BEH C_{18} particles, and the other is an open channel used for post column addition of the solvent. The PCA channel joins the separation channel prior to the emitter. In this application, ibuprofen metabolites in urine was used as an example to illustrate the enhanced sensitivity obtained by using post-column addition of isopropanol.

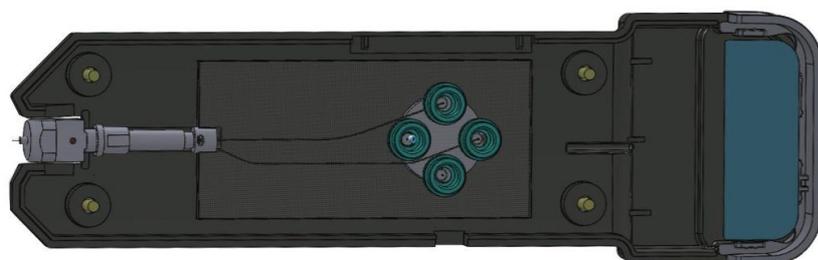


Figure 1. PCA iKey. The analytical channel is connected to the upper port and the post-column addition channel is connected to the right port.

Experimental

Sample preparation

Pre-dose urine and ibuprofen-metabolite-containing urine (3 hours after the oral administration of 200 mg) samples were collected from a healthy male volunteer. The urine samples were directly injected after a 1:50 dilution in water.

LC conditions

LC system:	ACQUITY UPLC M-Class (Figure 2)
Column:	PCA iKey BEH C_{18} Separation Device, 130Å, 1.7 μm , 150 μm x 100 mm (Figure 1)
Column temp.:	65 °C

Loop size:	1 μ L
Injection volume:	Full loop mode
Flow rate:	3.0 μ L/min
Mobile phase A:	10 mM ammonium acetate buffer pH 4.5
Mobile phase B:	Acetonitrile
PCA solvent:	Isopropanol
Flow rate PCA:	1.0 μ L/min
Weak needle wash:	10 mM ammonium acetate buffer pH 4.5
Strong needle wash:	50% acetonitrile, 25% methanol, 25% water
Seal wash:	90:10 water:acetonitrile

Gradient

Time (min)	Flow (μ L/min)	%A	%B	Curve
Initial	3	95	5	initial
10	3	35	65	6
11	3	5	95	6
14	3	5	95	6
15	3	95	5	6

MS conditions

MS system:	Xevo G2-XS QTof (Figure 2)
Ionization mode:	ESI negative
Capillary voltage:	2.3 kV
Source temp.:	150 °C
Cone voltage:	50 V
Source offset:	150 V
Quad profile:	Auto
Scan:	50-600 Da, 0.1 second
Data format:	Centroid
Analyzer mode:	Sensitivity
RF settings:	Auto



Figure 2. ionKey/MS system with ACQUITY UPLC M-Class System and Xevo G2-XS QTof.

Results and Discussion

The analysis of human urine in negative ionization mode using post-column addition of isopropanol showed a significant increase in sensitivity (Figure 3). The separation was performed using a 3 $\mu\text{L}/\text{min}$ gradient from 5 to 65% ACN in 10 minutes. Under standard gradient conditions, in the absence of isopropanol (Figure 3, top chromatogram), not all compounds were detected. Post-column addition of isopropanol allowed detection of more hydrophilic compounds, including ibuprofen metabolites. Additionally, adding isopropanol lowered the capillary voltage required for a stable electrospray, and therefore reduced the possibility of electric discharges that can generate undesired background noise.

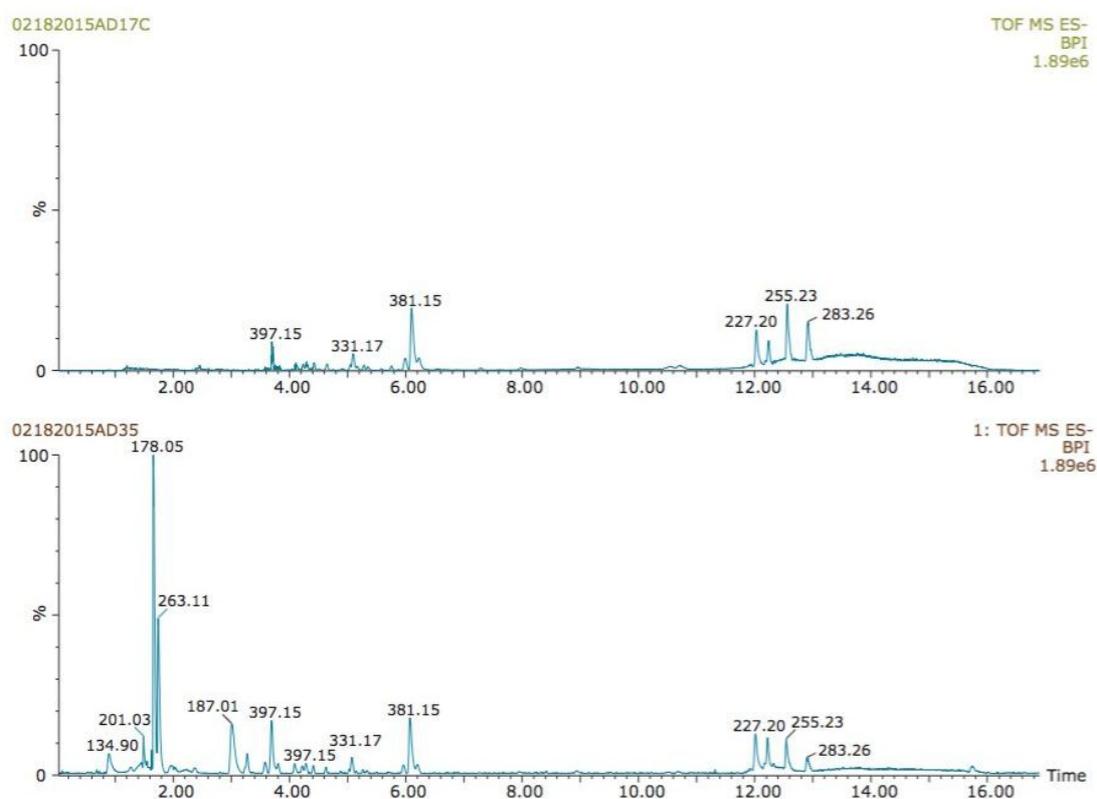


Figure 3. Chromatographic separation of human urine when 0 (top), and 1 (bottom) $\mu\text{L}/\text{min}$ IPA was used as a post-column modifier. The chromatograms are scaled to the highest relative intensity among the two chromatograms.

Figure 4 shows the chemical structure of ibuprofen and some of its major metabolites.

We examined the sample for evidence of ibuprofen metabolites using the combination of low (5 eV) and high

(25 eV) collision energies to generate both molecular ion and fragment ion data.

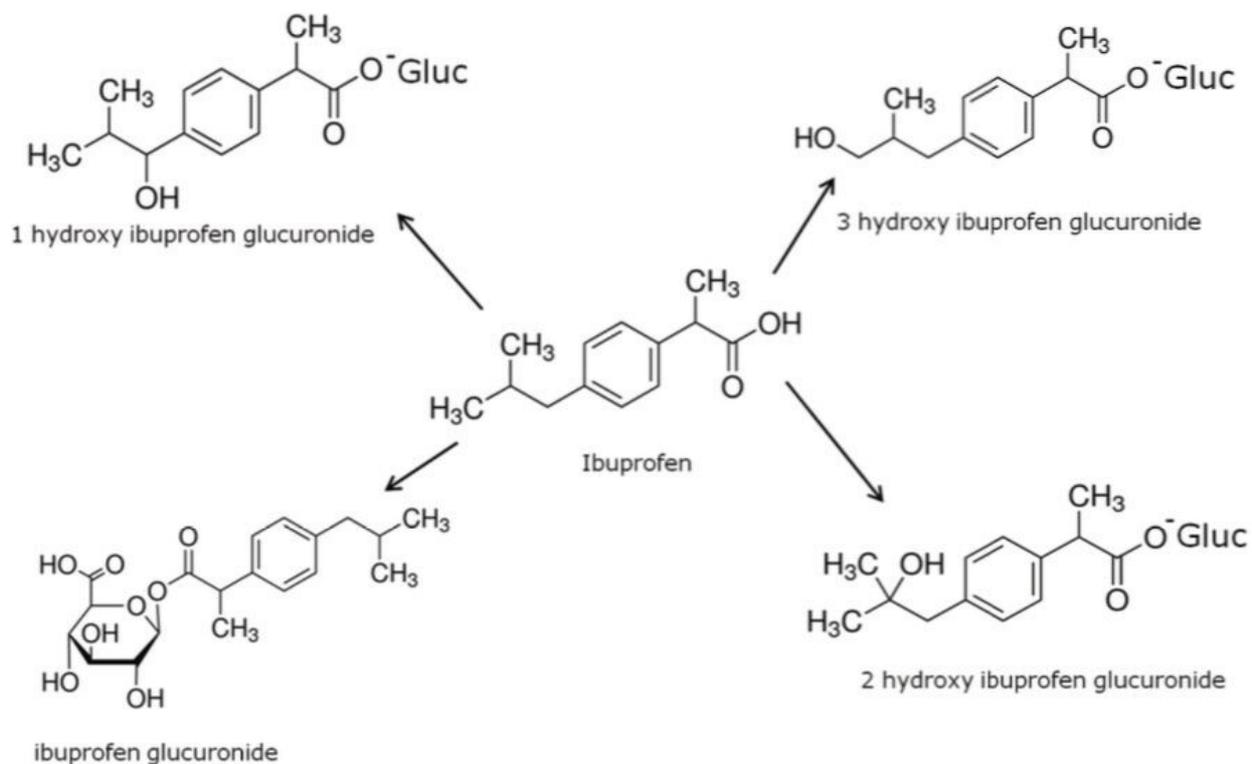


Figure 4. Ibuprofen and some of its associated metabolites.

The extracted ion chromatograms for m/z 397.1499, corresponding to hydroxylated glucuronide metabolites of ibuprofen are shown in Figure 5. For the most intense peak eluting with a retention time of 3.7 min, the signal increased by over 50% when IPA was used (Figure 5, bottom chromatogram).

In the absence of the post-column addition of isopropanol, analytes that elute in low %ACN were mostly undetected or produced very low signals.

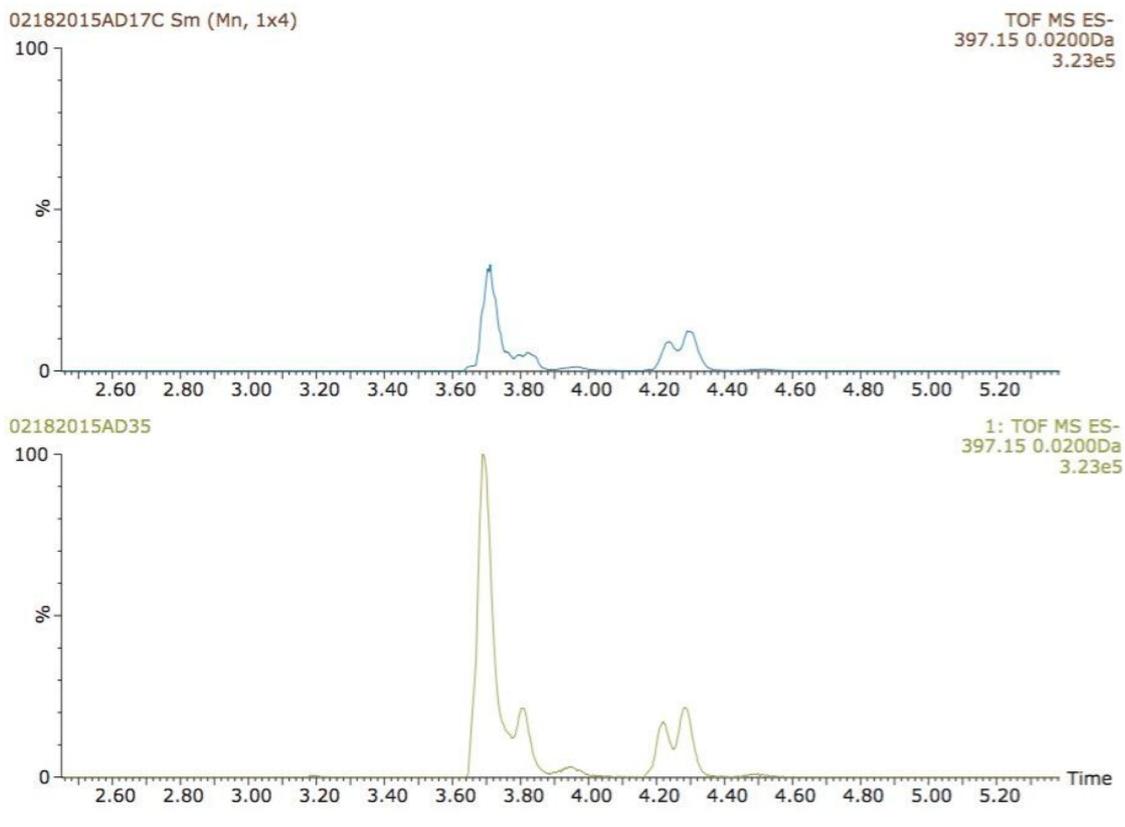


Figure 5. Extracted ion chromatograms of the hydroxylated glucuronide metabolites of ibuprofen; top chromatogram in the absence of IPA, bottom chromatogram with IPA.

As illustrated in figure 6, another common ibuprofen metabolite with an m/z of 411.1279, formed by the further oxidation of one of the side-chain-hydroxylated methyl group, was detected only when using post-column addition of isopropanol.

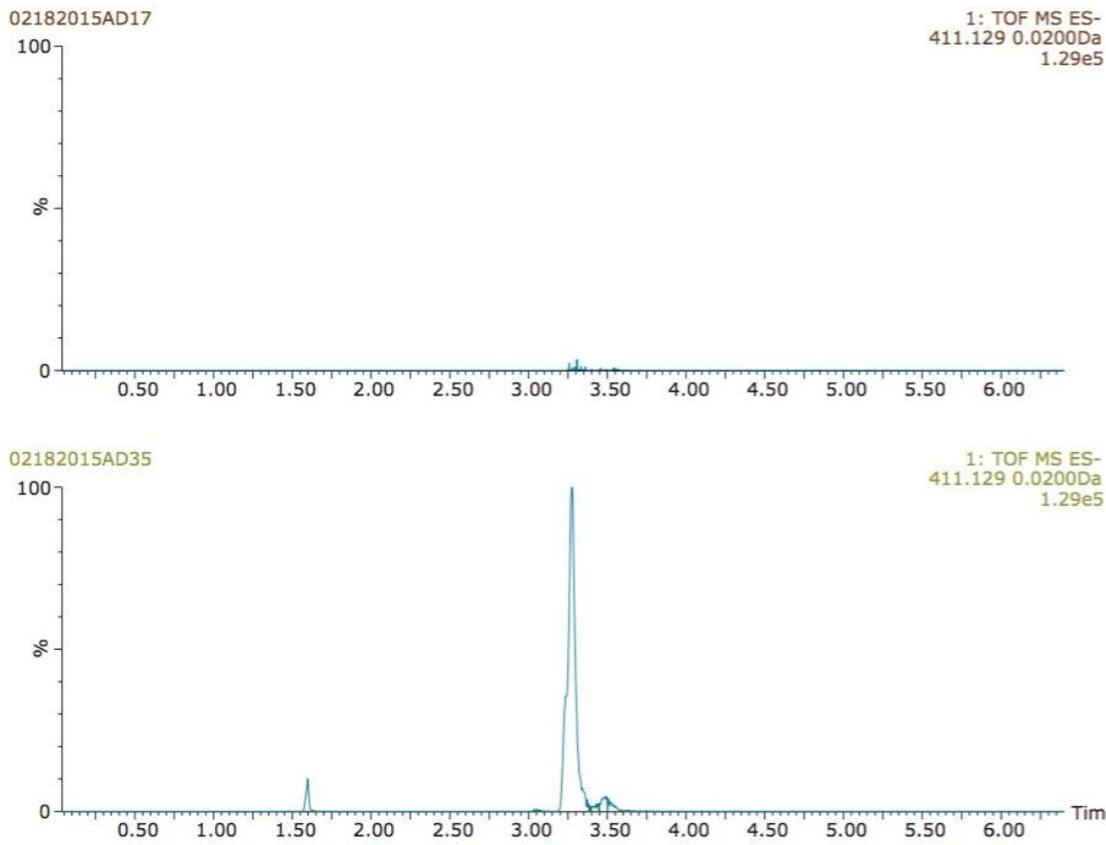


Figure 6. Extracted ion chromatograms of the ketone glucuronide metabolite of ibuprofen; top chromatogram in the absence of IPA, bottom chromatogram with IPA.

The advantage of this approach was further applied towards the detection of other urine metabolites. Hippuric acid ($m/z=178.0504$), an endogenous polar biomarker produced an intense signal in the presence of isopropanol, but was completely undetected under standard conditions (Figure 7).

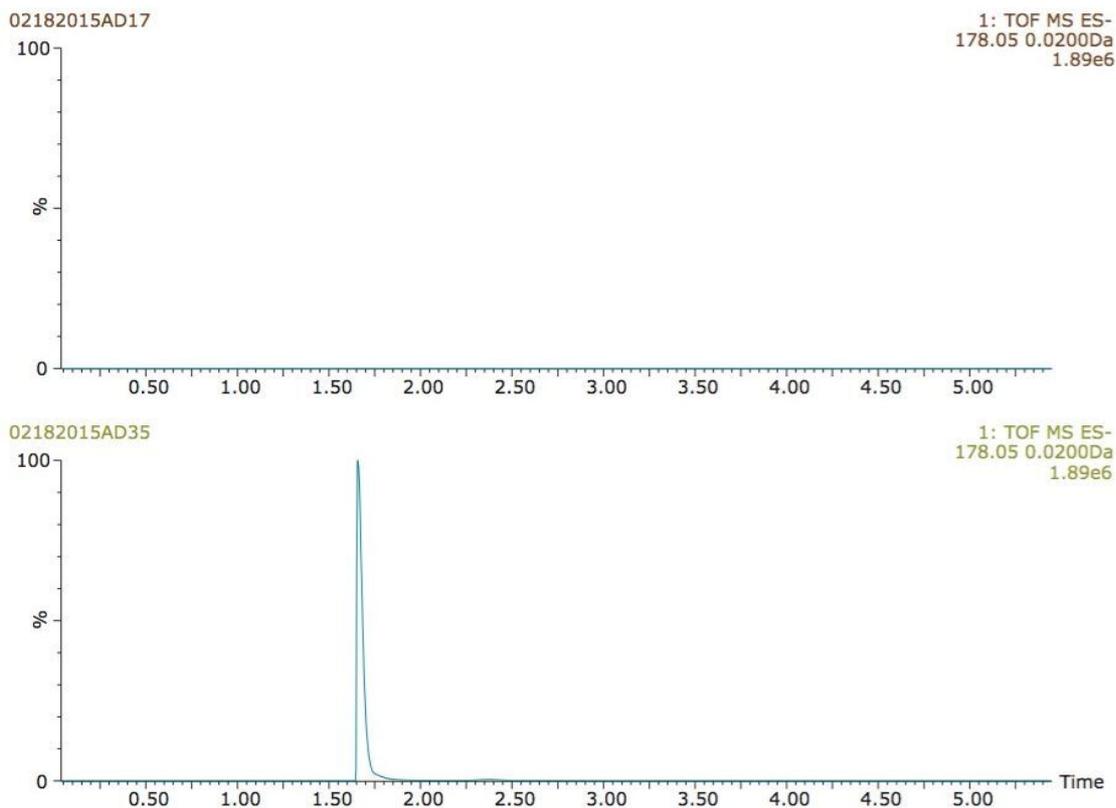


Figure 7. Extracted ion chromatograms of hippuric acid; top chromatogram in the absence of IPA; bottom chromatogram with IPA.

Conclusion

In low-flow negative-ion mode electrospray ionization, post-column addition of an organic solvent stabilized the electrospray and demonstrated improved sensitivity. Ibuprofen and associated metabolites, as well as hippuric acid, proved the utility of post-column addition to generate high-quality metabolic profiling data.

While this study is demonstrating the advantage of post-column addition in negative ionization mode, we foresee other applications where adding a post-column reagent can enhance the results.

References

1. Cech NB, Enke CG. Practical implications of some recent studies in electrospray ionization fundamentals. *Mass Spectrom Rev.* 2001 Nov-Dec;20(6):362–87.

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