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Comparative evaluation of ICP sample introduction systems to be used in the metabolite profiling of chlorine-containing pharmaceuticals via HPLC-ICP-MS

Balázs Klencsár[a], Carlos Sánchez[a,b], Lieve Balcaen[a], José Todolí[b], Frederic Lynen[c], Frank Vanhaecke[a,*]

[a] Ghent University, Department of Chemistry, Atomic & Mass Spectrometry – A&MS Research Unit, Campus Sterre, Krijgslaan 281-S12, 9000 Ghent, Belgium
[b] University of Alicante, Department of Analytical Chemistry, Nutrition and Food Sciences, P.O. Box 99, 03080 Alicante, Spain
[c] Ghent University, Department of Organic and Macromolecular Chemistry, Campus Sterre, Krijgslaan 281-S4-bis, 9000 Ghent, Belgium

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ABSTRACT

A systematic evaluation of four different ICP sample introduction systems to be used in the context of metabolite profiling of chlorine-containing pharmaceuticals via HPLC-ICP-MS was carried out using diclofenac and its major metabolite, 4′-hydroxy-diclofenac, as model compounds. The strict requirements for GMP validation of chromatographic methods in the pharmaceutical industry were adhered to in this context. The final aim of this investigation is an extension of the applicability and validatability of HPLC-ICP-MS in the field of pharmaceutical R&D. Five different gradient programmes were tested while the baseline peak width (w_b), peak capacity (P), USP tailing factor (A_s) and USP signal-to-noise ratio (USP S/N) were determined as major indicators of the chromatographic performance and the values obtained were compared to the corresponding FDA recommendations (if applicable). Four different ICP-MS sample introductions systems were investigated involving two units typically working at higher flow rates (~1.0 mL min^{-1}) and another two systems working at lower flow rates (~0.1 mL min^{-1}). Optimal conditions with potential for applicability under GMP conditions were found at a mobile phase flow rate of 1.0 mL min^{-1} by using a pneumatic micro-flow LC nebulizer mounted onto a Peltier-cooled cyclonic spray chamber cooled to −1 °C for sample introduction. Under these conditions, HPLC-ICP-MS provided a chromatographic performance similar to that of HPLC with UV detection. The peak shape (USP tailing factor = 1.1–1.4) was significantly improved compared to that obtained with the Peltier-cooled Scott-type spray chamber. Two alternative sample introduction systems – a POINT® and a High-Temperature Torch-Integrated Sample Introduction System (hTISIS) – were also tested at a flow rate of 0.1 mL min^{-1} using a chromatographic column with 1.0 mm ID. Although these systems allowed the peak shape to be improved compared to that obtained with the traditional Scott-type spray chamber, the limits of detection and of quantification achievable were strongly compromised due to the significantly lower sensitivity observed for Cl. In addition to a comparison of the aforementioned sample introduction systems, also the effect of spray chamber temperature was evaluated and it was demonstrated that proper temperature control plays an essential role in the optimization of HPLC-ICP-MS methods.

1. Introduction

Inductively coupled plasma – (tandem) mass spectrometry (ICP-MS(/MS)) is a well-known analytical technique for quantitative trace elemental analysis. Coupled with an adequate separation technique [1,2], *e.g.,* (ultra)high-performance liquid chromatography ((U)HPLC) [3–8], gas chromatography (GC) [9,10], capillary electrophoresis (CE) [11–13], ICP-MS(/MS) is also deployed in speciation studies, as a sensitive and element-specific detector. Due to its – at first sight – straightforward compatibility with (U)HPLC,

Abbreviations: ICP-MS(/MS), Inductively Coupled Plasma – (tandem) Mass Spectrometry; RP (U)HPLC, Reversed Phase (Ultra) High-Performance Liquid Chromatography; GC, Gas Chromatography; CE, Capillary Electrophoresis; ESI, Electrospray Ionization; HR, High Resolution; KED, Kinetic Energy Discrimination; GMP, Good Manufacturing Practice; ID, Internal Diameter; LOD, Limit of Detection; LOQ, Limit of Quantification; U.S. FDA, U.S. Food and Drug Administration; hTISIS, High-Temperature Torch-Integrated Sample Introduction System; UV, Ultraviolet detection.

* Corresponding author.
E-mail address: frank.vanhaecke@ugent.be (F. Vanhaecke).

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(U)HPLC-ICP-MS(/MS) also shows great potential in drug metabolite profiling, as has recently been reviewed by Klencsár et al. [2]. For a detectable hetero-element, ICP-MS(/MS) provides an analytical response that is independent of the chemical structure of the molecule in which the element is present, thus rendering the technique suitable for quantitative analysis, even without access to individual standards for each analyte of interest. This is an important advantage over other MS-based detection techniques, such as electrospray ionization-MS (ESI–MS). As ICP-MS is originally designed for the analysis of aqueous samples, several challenges accompany the hyphenation of reversed phase (RP) HPLC and ICP-MS, such as the handling of organic solvents and quantification issues owing to the use of gradient elution. Carbon depositions on the cones and torch can be avoided and stable plasma conditions can be maintained *via* the introduction of O_2 into the plasma when organic solvents are analysed. As with gradient elution, the matrix composition of the mobile phase is continuously changing, its use brings about continuously changing plasma conditions and thus, a continuously varying sensitivity for the target element throughout the chromatographic run. Of course, such changes in sensitivity must be avoided or need to be properly corrected for [2,6,8,14]. Strategies addressing this problem include quantification using online species-unspecific isotope dilution [6,14,15], the use of a compensation gradient [16–18] or mathematical correction [8,19,20]. The difficulties caused by spectral interferences, which are particularly pronounced in the case of light hetero-elements (*e.g.,* S, Cl, P) typically present in pharmaceuticals [2,21], cannot be overlooked either. Spectral interferences can be overcome by the application of higher mass resolution (HR) in sector field ICP-MS instruments [22] or by using kinetic energy discrimination (KED) and/or chemical resolution in the collision/reaction cell of quadrupole-based ICP-MS units [23,24]. The introduction of tandem ICP-MS instrumentation has rendered the latter approach much more powerful.

However, besides the capabilities of HPLC-ICP-MS(/MS) for sensitive detection and accurate quantification, demonstrated in multiple works already [3–8], also the chromatographic characteristics of these methods are of the utmost importance. Therefore, especially in the context of pharmaceutical analysis, where GMP validation is needed and strictly regulated by the Authorities, the chromatographic characteristics must be paid adequate attention to. It is, *e.g.*, self-evident, that additional dead volumes should be minimized in the HPLC-ICP-MS coupling to avoid peak broadening to the highest possible extent, rendering micro-flow LC nebulizers with lower ID preferable over the traditional nebulizers in this context. Also the volume and geometry of the spray chamber can have a significant impact on the peak broadening and peak shape, and thus indirectly on the accuracy, limit of detection (LOD) and limit of quantification (LOQ) achievable. An appropriate temperature control of the spray chamber (if control is possible) can also have an important effect on the figures of merit.

Taking the strict Authority requirements for the different system suitability parameters (*e.g.,* USP tailing factor should be $\leq$2 according to U.S. FDA [25]) and the fact that the chromatographic performance indicators also fundamentally determine the suitability and validatability of an HPLC-ICP-MS method into account, a systematic comparative study of different ICP sample introduction systems was carried through. The most critical chromatographic parameters were characterized using diclofenac and its major metabolite, 4'-hydroxy-diclofenac, as model compounds.

A Scott-type double-pass spray chamber, a cyclonic spray chamber, a POINT® sample introduction kit, recently introduced by Meinhard for the analysis of highly volatile organic solvents, and a total sample consumption system called High-Temperature Torch-Integrated Sample Introduction System (hTISIS) developed by Todolí et al. [26–28] were systematically evaluated for their use in HPLC-ICP-MS/MS using different gradient conditions. The figures of merit thus obtained were compared to the corresponding data for the HPLC-UV chromatograms, which were regarded as points of reference. The target compounds were monitored based on the Cl-atom they contain, using a state-of-art ICP-MS/MS system, as recently published by Klencsár et al. [7,8].

2. Experimental

2.1. Materials, stock and standard solutions

Diclofenac sodium (pharmaceutical secondary standard with a purity of 99.9%), 4'-hydroxy-diclofenac (analytical standard with a purity of $\geq$99.0%) and formic acid (purity $\geq$88.0%, *Trace*SELECT®) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (LC–MS grade) was purchased from VWR International (Leuven, Belgium). Ultra-pure grade water (resistivity $\geq$18.2 MΩ cm) was obtained from a Millipore Direct-Q water purification system (MQ-water, Millipore, Billerica, MA, USA). Ammonium chloride (1000 ± 2 mg L^{-1} for Cl) elemental stock solution applied for the optimization of the ICP-MS method was purchased from Inorganic Ventures (Christiansburg, VA, USA).

For the optimization of the ICP-MS/MS method, 1 mg L^{-1} Cl standard solution was prepared by dilution of ammonium chloride elemental stock solution with acetonitrile. Stock solution of diclofenac sodium was prepared by dissolving 21.1 mg of diclofenac sodium in 10 mL MQ-water and subsequently stored in the fridge at 2–8 °C. Stock solution of 4′-hydroxy-diclofenac was prepared by dissolving 1.15 mg of 4′-hydroxy-diclofenac in 500 μL acetonitrile and stored in the freezer at −20 °C. The standard solution containing the model compounds and used for the evaluation of different HPLC-ICP-MS/MS strategies was prepared by appropriate dilution of diclofenac and 4′-hydroxy-diclofenac stock solutions with acetonitrile – MQ-water 3–7 (v/v) mixture to obtain concentrations of 5.0 mg L^{-1} Cl as diclofenac plus 5.0 mg L^{-1} Cl as 4′-hydroxy-diclofenac.

2.2. ICP-MS/MS instrumentation

An Agilent 8800 "triple-quadrupole" ICP-MS/MS system (Agilent Technologies, Tokyo, Japan) was applied for the Cl-selective detection of the target compounds as $^{35}ClH_2^+$ in MS/MS mode with H_2 as a reaction gas in the octopole collision/reaction cell, as recently published by Klencsár et al. [7]. The method was tuned for maximum sensitivity for $^{35}ClH_2^+$ separately for the different sample introduction systems using a 1 mg L^{-1} Cl standard solution in acetonitrile, as mentioned above.

The instrument was equipped with a torch with a 1.0 mm ID injector tube (Agilent Technologies, Tokyo, Japan). Four different sample introduction systems were tested for HPLC-ICP-MS/MS purposes. A low internal volume PFA-LC nebulizer (Elemental Scientific, Omaha, NE, USA) was combined with (i) a Peltier-cooled Scott-type spray chamber (Agilent Technologies, Tokyo, Japan), (ii) a PC^3 Peltier-cooled cyclonic spray chamber (Elemental Scientific, Omaha, NE, USA) and (iii) a High-Temperature Torch-Integrated Sample Introduction System (hTISIS), developed by Todolí et al. [28–30]. The 9 mL single-pass spray chamber of hTISIS typically works at a higher temperature, depending on the solvent from room temperature to 400 °C, to ensure quantitative analyte transport into the plasma. Therefore, only low sample flow rates can be used to avoid overloading of the ICP. The hTISIS was operated in continuous sample aspiration mode and the spray chamber was heated to 150 °C during the analysis.

Additionally, also a (iv) POINT® sample introduction kit (Meinhard, Golden, CO, USA) was tested. The POINT® kit is a miniaturized