

# Potential of Liquid Chromatography–Atmospheric Pressure Chemical Ionisation Tandem Mass Spectrometry for Determination of Fosetyl-aluminium Residues in Dried Hops

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## Abstract

POUSTKA J., HAJŠLOVÁ J., HOLADOVÁ K., NOVÁKOVÁ K. (2004): **Potential of liquid chromatography–atmospheric pressure chemical ionisation tandem mass spectrometry for determination of fosetyl-aluminium residues in dried hops.** Czech J. Food Sci., 22: 24–28.

New analytical procedure for the determination of fosetyl-aluminium in hop cones (also called “hops”) was developed. Liquid chromatography–atmospheric pressure chemical ionisation tandem mass spectrometry (LC–APCI–MS/MS) allowed a highly selective determination of this fungicide which facilitated the sample preparation. The sufficiently low detection limit (1 mg/kg of dried hops) with the acceptable method performance characteristics (RSD 9%, recovery 76% – at the level of 10 mg/kg) complies with the need to inspect the observance of maximal residual limit 100 mg/kg. The application of LC–MS technique thus provided an effective way of fosetyl-aluminium determination in the complex hops matrix.

**Keywords:** fosetyl-aluminium; pesticide residues; hops analysis; liquid chromatography; mass spectrometry

Fosetyl-aluminium (Figure 1) is the active ingredient of some pesticide formulations (e.g. Aliette or Fosim) widely used to control plant diseases caused by fungi of *Phytophthora* genus and also by several bacterial plant pathogens (TOMLIN 2000–2001; O’NEILL *et al.* 2002; MOLLER *et al.* 2003; BARRETT *et al.* 2003). In accordance with the requirements of legislative regulations (Decree No. 465/2002 Collection of Laws, the Czech Republic), the control

of fosetyl-aluminium residues in treated crops such as hop cones should be carried out. Fosetyl-aluminium is a very polar compound ( $pK_{ow} = 2.7$ ), which complicates its determination in complex plant matrices with respect to its separation and detection. In water solutions, fosetyl-aluminium dissociates to fosetyl which thus becomes the target analyte.

The use of gas chromatography (GC) is necessarily accompanied by an appropriate derivatisation step prior to the instrumental determination. The method based on gas chromatography coupled with flame photometric detection (GC–FPD) as described in the Dutch manual for the pesticide residue analysis converts extracted fosetyl to the methylated derivative (Analytical Methods 1988). It

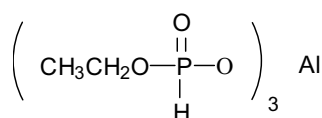


Figure 1. Structure of fosetyl-aluminium

should be noted that this fairly laborious procedure was developed for the analysis of chicory. Without modifications, it is not applicable for the examination of hops extracts which are typically abundant in matrix components. Their separation from the target analyte by the common clean-up procedures is complicated; consequently, the accuracy of GC analysis may be poor because of multiple matrix effects. Other available methods enabling the direct determination of fosetyl-aluminium utilise ion exchange chromatography coupled with electrochemical detection (GIORDANO *et al.* 1995), microcolumn liquid chromatography or capillary electrophoresis coupled with flame photometric detection (HOOIJSCHUUR *et al.* 2001). A screening method based on microbioassay (KUHAJEK *et al.* 2003) was also developed. As regards hops analysis, the application of some of the above mentioned liquid-phase based methods was not possible because of the lack of the appropriate instrumentation. Therefore, the available LC-MS technique, which had already been used for other polar pesticides analysis (ZROSTLÍKOVÁ *et al.* 2002, 2003), was optimised for the fosetyl-aluminium determination.

## MATERIALS AND METHODS

**Chemicals.** Deionised water was prepared using Milli-Q water purification system (Millipore, USA), methanol (HPLC gradient grade) was purchased from Merck (Germany), ammonium formate (99.8%) was provided by Sigma-Aldrich (USA). The standard of fosetyl-aluminium (99.5%) was supplied by Rhone-Poulenc Agrochimie (France).

**Calibration standards.** Stock solution of fosetyl-Al in water was prepared in concentration 1 mg/ml. By appropriate dilution, a set of standard solutions in water was obtained and used for calibration (0.5, 1.0, 5.0, 10, 50, 100, 500 µg/ml). Also, standard solutions in the rough hops extract and in the extract processed by solid phase extraction (SPE) were prepared by spiking with corresponding water solutions. In the first case – filtered extract (0.1 g of original matrix/ml) was spiked, in the second case – SPE processed extract (0.5 g of original matrix/ml) was spiked. For the preparation of these matrix matched standards, hops samples untreated with fosetyl-aluminium were used.

**Sample preparation.** 2 g of dried, finely ground hops cones were weighted into 50 ml beaker and

soaked with 20 ml of water. After 30 min, the beaker was placed into ultrasonic bath for 5 min. The extract was filtered using Büchner funnel under vacuum and the filtrate was collected into 20 ml volumetric flask and made up with water. 10 ml of the rough extract was filtrated using 5 µm Millipore PTFE filter and passed through the SPE column Supelclean LC-SAX 3 ml Tubes (Supelco, USA). After SPE column vacuum drying (5 min), the elution of fosetyl-aluminium was carried out with 2 ml of 0.1M ammonium formate water solution. The final eluate was made up to 2 ml in a volumetric flask and transferred to a vial.

**LC-MS system.** HPLC HP 1100 binary system equipped with GROM SAX column 150 × 4 mm (10 µm) coupled with guard column 20 × 4 mm (GROM, USA) was used for the separation of the sample components. Mobile phase consisted of mixture of methanol:100mM ammonium formate in water (50:50, v/v), flow rate was 0.5 ml/min. The injected volume was 40 µl.

LCQ Deca Finnigan MS system was operated in positive APCI mode at sheath gas flow rate 1.2 l/min, auxiliary gas flow rate 3 l/min, capillary temperature 200°C, discharge current 5 µA (corresponding to 4.5 kV approx.). During the analysis two signals were recorded: 1. MS mode – range  $m/z = 50-250$ , 2. MS/MS mode: daughter ion  $m/z = +111$  originating from parent ion  $m/z = +128$ .

## RESULTS AND DISCUSSION

The determination of fosetyl-aluminium residues in hop cones is rather a difficult analytical task. Owing to the acidic nature of fosetyl (dissociation product of fosetyl-aluminium in water solution), the use of anion exchange LC column was chosen. Under conventional experimental conditions, the release of analytes possessing as low  $pK_a$  value as fosetyl (0.8) from stationary phase by a competition with stronger acid anions could be obtained. However, the use of concentrated organic acids such as formic acid or trifluoroacetic acid can cause damage to MS system. Therefore, suitable ammonium salt was used creating ion pair (ammonium formate) which has a weak affinity to the stationary phase. This approach ensured an adequate chromatographic behaviour of fosetyl and also enhanced ionisation process in APCI-MS interface. Moreover, formation of  $[M+NH_4]^+$  adduct parent ions ( $m/z = +128$ ) provided secondary intensive  $[M+1]^+$  ion ( $m/z = +111$ ) in MS/MS

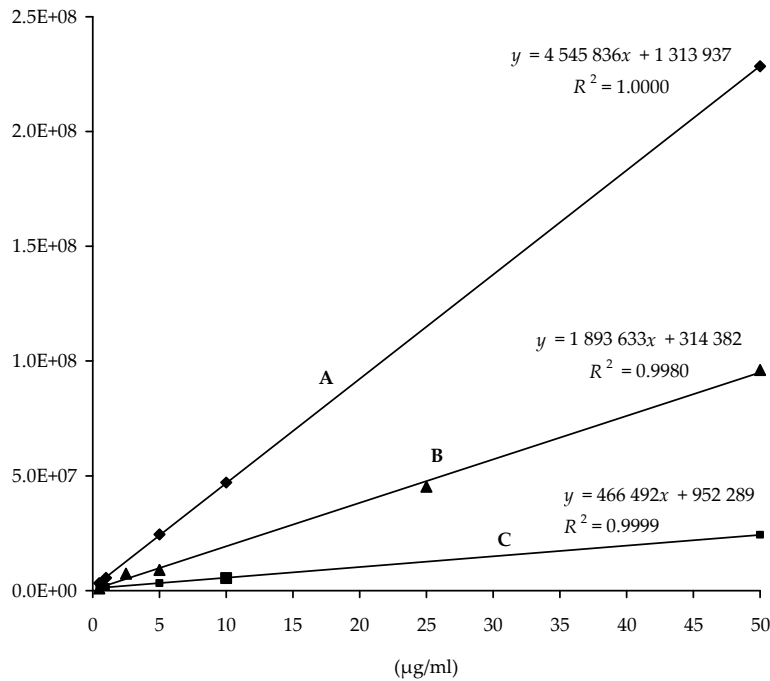


Figure 2. Comparison of different procedures of calibration. A – (i) water standard solutions, B – (ii) the SPE processed extracts spiked with water standard solutions, C – (iii) the rough extract spiked with water standard solutions (see Materials and Methods – Calibration standards)

mode, which facilitated fosetyl confirmation and quantification.

Experimental measurements proved significant differences among three sets of calibration, which were based on (i) water standard solutions, (ii) the SPE processed extracts spiked with water stand-

ard solutions, and (iii) the rough extract spiked with water standard solutions (Figure 2). These results proved the necessity to use the calibration based on the cleaned extract spiked with standard solutions to minimise under(-over)estimation of fosetyl-aluminium content caused by coextracted

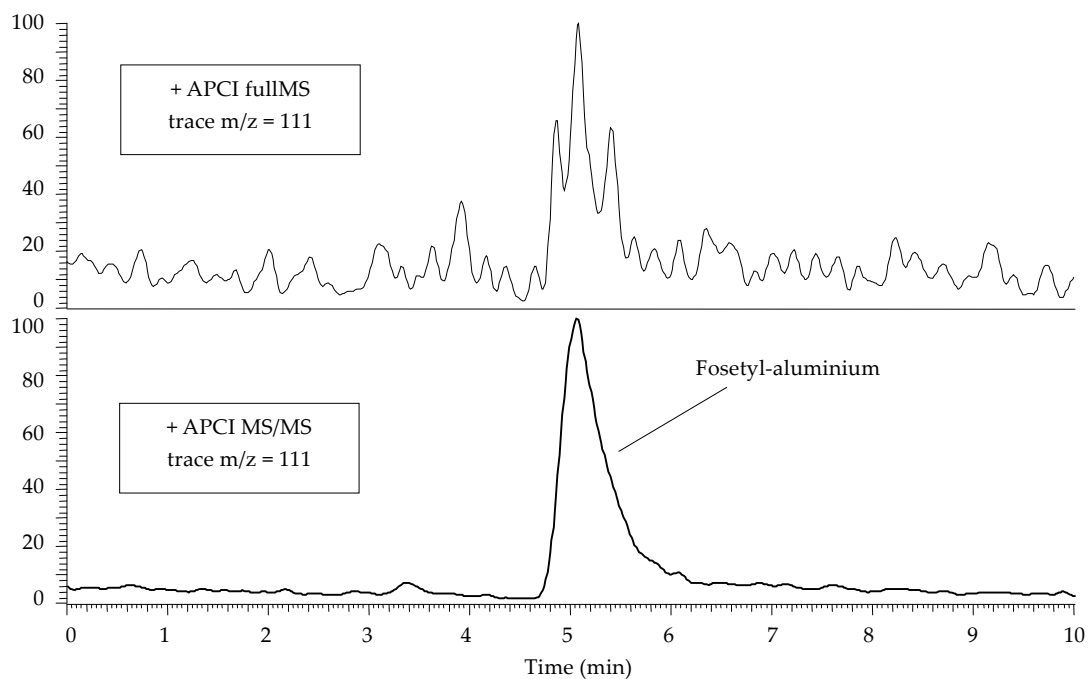


Figure 3. LC-MS analysis of fosetyl-aluminium in the spiked hops extract processed by SPE at concentration 50 µg/ml

matrix components. The differences in chemical noise are apparent from the chromatograms (Figure 3). By the upper chromatogram, the lower selectivity of MS mode is illustrated. The signal for ion at  $m/z = +111$  is very close to noise, while in the highly selective MS/MS mode (lower chromatogram), a significant fosetyl-aluminium peak is visible.

One of the most difficult parts of the analytical procedure optimisation is the verification of the extraction efficiency. Because of the lack of certified reference materials of hop containing fosetyl-aluminium, the commonly used method in this field (HENGEL & SHIBAMOTO 2002), which is based on the direct sample spiking just before extraction, was used for the recovery testing. As regards the recovery attained, the processing of the spiked rough extract by SPE procedure provided an acceptable value – 76% (at the level of 10 mg/kg). Repeatability expressed as RSD = 9% at the level of 10 mg/kg for the purified extract was acceptable for the tested matrix/analyte combination. The estimated limit of detection (1 mg/kg) and the limit of quantification (5 mg/kg) are fairly below maximum residue limit (100 mg/kg) for fosetyl-aluminium in dried hops established in the Czech Republic.

### Conclusions

The application of LC-MS in combination with ion exchange chromatography significantly facilitates the sample preparation procedure in comparison with GC-based methods and can be a suitable alternative to the use of electrochemical detection or to the special combination of liquid phase – flame photometric detection. A weak point of all techniques available is the lack of the appropriate reference material which is necessary for the method validation. On the other hand the results attained correspond with the “state of the art” in the polar pesticides residues analysis in hops.

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Received for publication October 16, 2003

Accepted November 24, 2003

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**Souhrn**

POUSTKA J., HAJŠLOVÁ J., HOLADOVÁ K., NOVÁKOVÁ K. (2004): **Možnosti kapalinové chromatografie–tandemové hmotnostní spektrometrie s chemickou ionizací za atmosférického tlaku při stanovení reziduí fosetyl-aluminia v sušených chmelových šišticích.** Czech J. Food Sci., 22: 24–28.

Byl vyvinut nový analytický postup pro stanovení fosetyl-aluminia v chmelových šišticích. Aplikace kapalinové chromatografie–tandemové hmotnostní spektrometrie s chemickou ionizací za atmosférického tlaku (LC–APCI–MS/MS) umožňuje vysoce selektivní stanovení tohoto fungicidu a také zjednodušuje přípravu vzorku. Dostatečně nízký limit detekce (1 mg/kg sušených chmelových šištic) a přijatelné pracovní charakteristiky (RSD 9 %, zpětná výtěžnost 76 % na hladině 10 mg/kg) odpovídají potřebě kontrolovat dodržování maximálního limitu reziduí fosetyl-aluminia ve chmelu (100 mg/kg). Zavedení LC-MS techniky přineslo efektivní způsob stanovení fosetyl-aluminia ve složité chmelové matici.

**Klíčová slova:** fosetyl-aluminium; reziduální analýza; analýza chmelu; kapalinová chromatografie; hmotnostní spektrometrie

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