New HPTLC/ESI-MS method for caffeine quantification using SIDA

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Scope

The flexibility of planar chromatography regarding number of samples analyzed simultaneously (up to 36), application volumes (nL to mL), and multiple detection (UV, Vis, FLD) together with less sample clean-up in comparison with other chromatographic techniques, have provided it a privileged position amongst the analytical tools for high-throughput analysis. In the last years the hyphenation with mass spectrometry (MS) is gaining interest due to the increase of useful interfaces to couple HPTLC with MS. Luftmann [1] developed a plunger-based device with a simple operation without requiring special equipments or adjustments. Considering these features, in our previous works this interface was enhanced to be used with glass plates and well proven for qualitative analysis of food [2] and pharmaceuticals [3] by HPTLC/MS at ng levels. Continuing these approaches, the objective of this work was to evaluate the improved plunger-based device (Fig. 1) for quantitative analysis of food and pharmaceutical products by HPTLC/ESI-MS using stable isotope dilution analysis (SIDA).

Results and discussion

The use of an internal standard nullified the dispersion caused by the plunger manual positioning (Fig. 2 and 3), thus a highly reliable method according to the validation results (Table 1) was obtained. HPTLC/SIDA-ESI-MS results were comparable with those obtained by validated HPTLC/UV methods (Table 2).

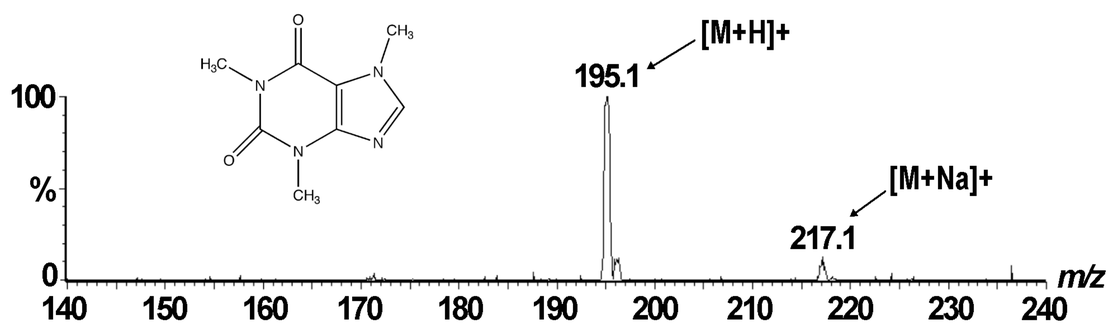

Figure 2. Caffeine mass spectrum obtained by HPTLC/ESI-MS showing the protonated molecule at *m/z* 195 and the sodium adduct at *m/z* 217.

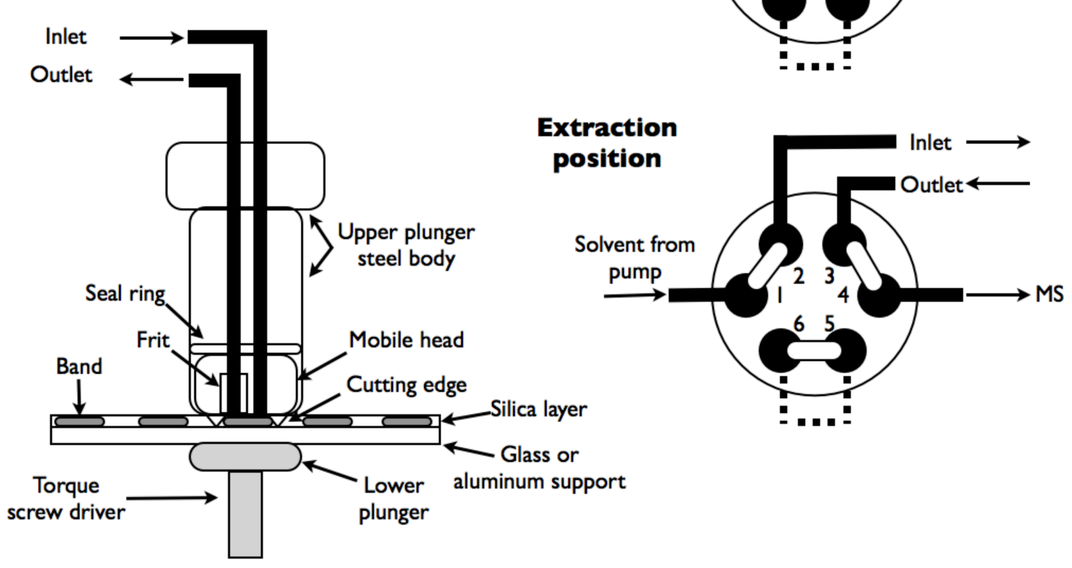

Figure 1. Scheme of the plunger-based extractor depicting the interface components and the two positions of the six-port valve.

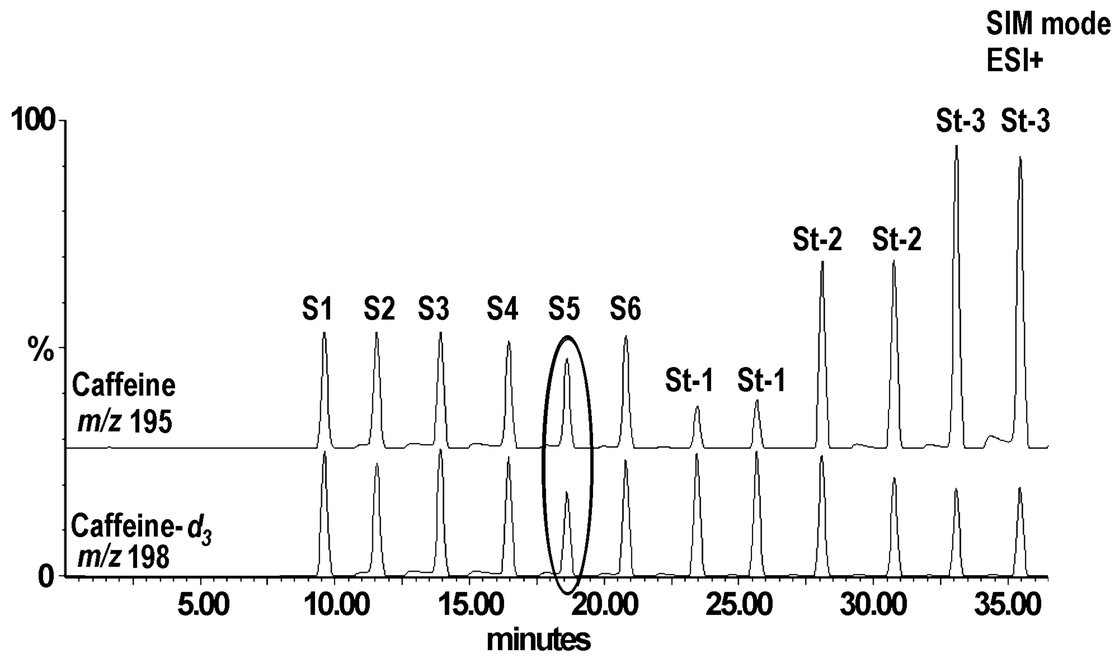

Figure 3. Overlayed mass chromatograms of caffeine and caffeine-d_3, describing the elution profile of samples (S1 to S6), caffeine standard (St-1=50 ng, St-2=300 ng and St-3=500 ng) and caffeine-d_3 as internal standard (100 ng). Slight differences due to the manual positioning were corrected by the internal standard (see S5) [4].

Table 1. Validation results according to ICH [4]

Parameters	
Linear range (*n*=3)	50-500 ng
R^2	0.9998
Repeatability (*n*=6, RSD)	3.75%
Intermediate precision (*n*=2, RSD)	0.68-8.60%
Limit of detection (S/N 3)	0.75 ng/band
Limit of quantification (S/N 10)	2.50 ng/band

Table 2. Caffeine quantification by HPTLC/ESI-MS and HPTLC/UV

Parameters	Caffeine ng/band	
	Pharmaceutical mean[a] ± SD[b]	Energy drinks mean[a] ± SD[b]
HPTLC/ESI-MS	99.82 ± 3.75	338.09 ± 4.87
RSD[c] (%)	3.75	1.44
HPTLC/UV	104.74 ± 1.51	334.86 ± 5.63
RSD[c] (%)	1.44	1.68

[a] *n*=6 [b] standard deviation [c] relative standard deviation

Conclusion

Major features of this simple interface are evident: it can universally be connected to all LC-MS systems without any adjustment or mass spectrometer modification. Detectability is given even at the picogram level on normal phases. It can be used for all plate sizes and carriers. The precision (%RSD) obtained was ~10% without internal standard and 4 % with internal standard. Thus it can be considered as one of the most reliable and universal interfaces for HPTLC/MS.

Acknowledgements
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References:
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2. M. Aranda and G. Morlock (2006) J. Chromatogr. A 1131, 253-260
3. M. Aranda and G. Morlock (2007) J. Chromatogr. Sci. 45, 251-255
4. M. Aranda and G. Morlock (2007) Rapid Commun. Mass Spectrom. 21, 1297-1303