Combining selectivity from chromatography and fast ion separation by MS in bioanalysis: simultaneous determination of aspirin and salicylic acid in limited volume plasma samples by ESI(-)-LC-MS/MS

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1. Introduction and Aim

Today's oldest known prescription drug, aspirin, still has a widespread use e.g. as an anti-inflammatory and pain relief agent, also in the eastern African region. However, questions have arisen on the medium term stability (storage under tropical conditions), and thus bioavailability of aspirin tablets present on the African market. Indeed, in vitro dissolution testing (USP 24) revealed poor drug release for some African formulations (1). Our aim was to develop a quantitative method for the simultaneous determination of aspirin (ASA) and salicylic acid (SA) in limited volume plasma samples using LC-MS/MS, in view of the subsequent bioequivalence testing of some oral ASA formulations.

Although seemingly an old and well known drug, ASA presents many analytical challenges. It is extremely labile in solution, preventing exhaustive sample clean-up procedures, and hard to reconcile with the selectivity of MS/MS as it shows extremely poor ionisation (ESI and APCI) in either negative or positive ion mode.

2. Experimental

Materials and methods:

- ASA, SA and 3-methyl-SA (IS) were obtained from Sigma-Aldrich (Bornem, B). All solvents used were of HPLC-grade and obtained from Merck (Darmstadt, D). Other products were of analytical grade (ammonia, formic acid (FA), acetic acid (AA), sulphuric acid, sodium sulphate and sodium tungstate).
- Analytical set-up: a 2695 Alliance LC chromatographic system (Waters) coupled to a triple quadrupole MS instrument (Quattro Ultima, Waters-Micromass MS Technologies, UK) with the MS interface used in the electrospray neg. ion mode (ESI(-))
- Stock and calibration solutions: at -20°C, protected from light, used no longer than one month, stock concentrations: for ASA and SA, 1.25 mg/mL, for the IS, 1 mg/mL
  SA and IS: in AcCN
  ASA: in AcCN, dried on sodium sulphate for 48 h and complemented with 2% FA
  → stability for one month (decomposition of ASA to SA)

Sample clean-up and LC-MS conditions:

- Fast "one-tube" approach
  250 µL of crude plasma + 10µL of sulphuric acid (residual pH 1)
  + 20 µL IS-solution (0.1 mg/mL)
  + 40 µL of AcCN (cal. standards: spiking of ASA, SA)
  + 50 µL of a 10% sodium tungstate solution (protein ↓)
  Vigorous shaking (15 s) and centrifugation (6000 rpm, 10 min) yielded a clear and protein-free supernatant; 15 µL was injected onto the chromatographic system.
- Chromatography:
  - Analytical column: XTerra MS C_8 column (Waters, 3.5 µm p.s., 150*2.1 mm)
  - Guard column: Hypersil BDS (Alltech, 3.5 µm p.s., 20*2.1 mm)
  - Total runtime: 15 min
  - Mobile phase: flow rate 0.2 mL/min, gradient of water and AcCN (ratio 9:1) complemented with 0.02% acetic acid (pH 3.1)
- MS/MS:
  - ESI (-), MRM
  - quantitation based on peak area ratios (ASA/IS and SA/IS)
    m/z 137.0 to 93.1 for ASA and SA
    m/z 151.0 to 107.0 for the IS

Table 1: Overview of the applied ESI (-) MS/MS conditions

Compound	Precursor ion Ion	*m/z*	Product ion *m/z*	Cone voltage (V)	Collision energy (eV)
ASA	$[M-H]^-$	137.0	93.1	30	18
		179.0	137.0		
SA	$[M-H]^-$	137.0	93.1	30	18
		137.0	65.1		
IS (3-MeSA)	$[M-H]^-$	151.0	107.0	30	16
		151.0	95.0		

Due to the chemical instability of ASA (basic hydrolysis of the ester-function), it is of prime importance that sample preparation and chromatographic separation are achieved under acidic conditions. Nevertheless, as ASA and SA are both acidic compounds, they need to be analysed in the ESI negative ion mode. Therefore post-column decomposition of ASA to SA was applied inside the LC-tubing leading the column eluent to the mass spectrometer. To that end, a 0.5 M ammonia solution was added to the column eluent, via a T-piece immediately behind the analytical column and using an auxiliary pump (pumping rate 50 µL/min). As a result, 250 µL/min of eluent with a pH of 10 entered the ESI interface.

3. Method validation

Investigated bioanalytical parameters: linearity, precision, accuracy, selectivity and sensitivity. Results are summarised in Table 2.

Table 2: Method validation data

		ASA			SA		
		0.2	10 µg/mL	100	0.2	10 µg/mL	100
Total precision (n = 10) %CV		4.4	6.7	1.2	7.3	6.0	1.2
Within-day precision (n = 5) %CV		3.4	5.5	1.0	6.2	5.3	0.9
Accuracy (n = 10) % of nominal		101.5±3.5	94.5±5.2	102.3±1.0	98.9±6.1	101.5±5.4	100.63±0.9
Linearity	Weighing: 1/x		Y = bX + c			Y = aX² + bX + c	
	Range (µg/mL)		0.1 - 100			0.1 - 100	
	Curvature (a)		-			-0.000152	
	Slope (b)		0.061			0.073	
	Intercept (c)		-0.002			0.006	
	R		0.9989			0.9996	
LOD (µg/mL)			0.10			0.1	
LOQ (µg/mL)			0.08			0.06	

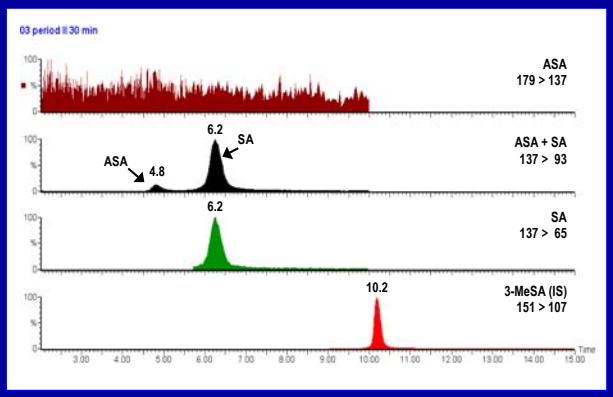

Figure 2: Extracted Ion Chromatograms of a real patient plasma sample, 30 min after ingestion of a 300 mg ASA tablet. The transition *m/z* 179 to 137 was monitored to check for complete decomposition of ASA to SA, prior to entrance into the MS.

4. Results

The suitability of the deloped analytical approach was demonstated in light of bioequivalence testing in healthy human volunteers of two tablet formulations, containing a single dose of 300 mg ASA, Dispril® (Reckitt & Coleman) and a product of Shelys Pharmaceutical Industries available on the Tanzanian market (Figure 2). Results indicate that, in vivo, there is no statistical difference between both formulations (Figure 3).

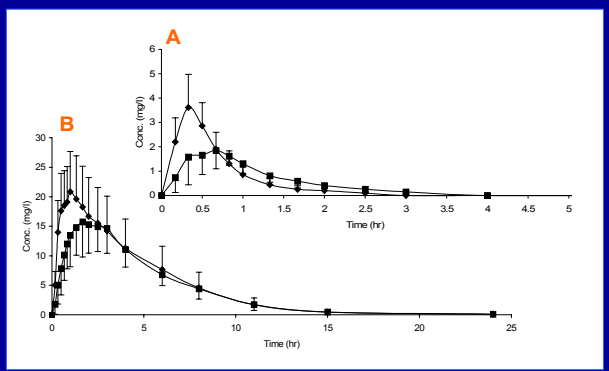

Figure 3: Mean (n=10) ASA (A) and SA (B) plasma concentration/time profiles following administration of a single dose of 300 mg acetylsalicylic acid: Dispril® (◆); Shelys formulation (■).

5. References and acknowledgements

(1) Risha P.G., Influence of dissolution rate on the bioanailability of two acetylsalicylic acid tablet formulations, Ph.D. thesis, chapter 5 (2003).

The authors would like to thank W. Goeteyn for his technical assistance. This work was supported by grant …….