LABORATORIO DE ANÁLISIS
DR. ECHEVARNE

IMPROVEMENT OF A METHOD TO DETERMINE 5-AMINOSALICYLIC ACID (5-ASA) AND N-ACETYL-5-AMINOSALICYLIC ACID (N-A-5-ASA) IN PLASMA SAMPLES (HPLC-FLD VERSUS LC-MS/MS)

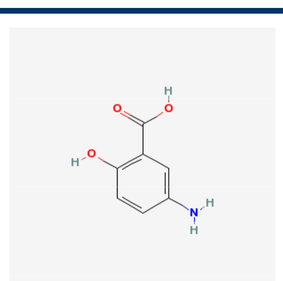
AUTHORS: González-Haro S., Herrera-García D., de Prada-Sánchez T., Rodríguez-Larena M.C., Sevillano-Serrat V., Soler-Gumbau E., Pelagio-Brualla P.(*)
(*)e-mail: ppelagio@echevarne.com

INTRODUCTION

A method for the analysis of 5-aminosalicylic acid (5-ASA) and its metabolite, N-Acetyl-5-aminosalicylic acid (N-A-5-ASA), in plasma samples using LC-MS/MS has been developed and validated in our laboratory to improve the method which has been previously been used for years with HPLC-FLD.
Due to the high polarity of these molecules, its analysis is not an easy task: in the old method was necessary to carry out solid phase purification with MCX cartridges and an Ion-Pair chromatography. The newly developed method is a great improvement from an analytical point of view: the ion pair chromatography is substituted by an HILIC column and the detection is carried out with the selectivity and sensitivity of an API-4000 MS/MS. The time of each chromatogram is reduced from 15 min to 5 min.

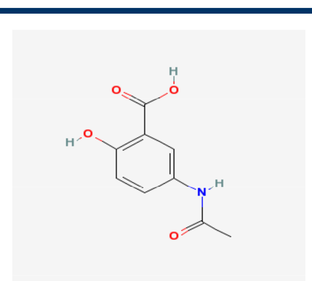
DRUG

5-Aminosalicylic acid (5-ASA or mesalazine), is an anti-inflamatory drug used to treat inflamation of the digestive tract ulcerative colitis and mild to moderate Crohn's disease. N-A-5-ASA is the main active metabolite of 5-ASA. On the other hand, 5-aminosalicylic acid (5-ASA) is considered a primary metabolite of sulfasalazine, represents the therapeutic active moiety of it.



5-ASA

Mol. Mass: 153.14 g/mol



N-A-5-ASA

Mol. Mass: 195.17 g/mol

LC-MS/MS METHOD

Extraction: Protein Precipitation

1. Add 0.1 mL of plasma from each sample.
2. Add 5 µL of internal standard (3-amino-4-methylbenzoic acid).
3. Add 400 µL of acetonitrile.
4. Mix with vortex for 10 seconds.
5. Centrifuge at least 3000 r.p.m., 5 minutes.
6. Transfer approx. 400 µL of the organic layer to a wellplate.

Chromatography and Detection

Column:	Atlantis™ Hilic Silica, 150 × 4.6 mm, 5 µm
Flow:	1 mL/min
Program (5 min):	20 mM acetic acid solution (30%) Acetonitrile (70 %)
Injection volume:	10 µL
Column temperature:	25ºC
Autosampler temperature:	4ºC
Spectrometer:	API 4000
Detection (MRM):	ESI (-)
Ion monitoring:	5-ASA: m/z 152.0>108.2
	N-A-5-ASA: m/z 194.0>107.1
	ISTD: m/z 150.0>105.9

HPLC-FLD METHOD

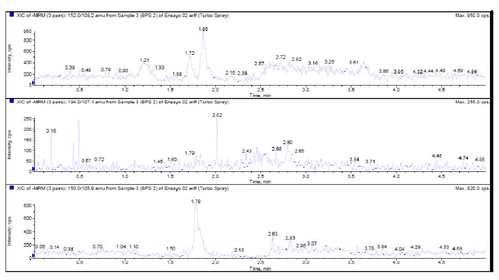
Extraction: Solid-phase by cartridges centrifugation

1. Add 0.3 mL of plasma from each sample.
2. Add 5 µL of internal standard (3-amino-4-methylbenzoic acid)
3. Add 50 µL of 1N hydrochloric acid solution.
4. Add 400 µL of water.
5. Mix with vortex for 2 seconds.
6. Activate OASIS MCX ™ cartridges with 1 mL of methanol.
7. Condition OASIS MCX™ cartridges with 1 mL of water.
8. Apply each sample to the corresponding cartridge.
9. Rinse 1 mL of 0.1 N hydrochloric acid solution.
10. Elute with 1 mL of methanol.
11. Elute with 1 mL of 5% ammonia solution in methanol.
12. Evaporate to dryness under a steady stream of nitrogen N2 (35ºC).
13. Reconstitute with 100 µL of Pic™ A solution.
14. Mix with vortex for 30 seconds.
15. Centrifuge at least 3000 r.p.m., 5 minutes.
16. Transfer the extract to a wellplate.

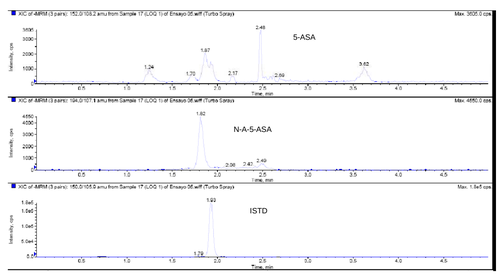
Chromatography and Detection

Column:	LUNA™ C18 (2), 150 x 4.6 mm, 5 µm
Flow:	1 mL/min
Program (15 min):	PIC A™ en H_2O (70 %) Methanol (30 %)
Injection volume:	15 µL
Column temperature:	25ºC
Autosampler temperature:	25ºC
FLD detector:	λ excitation [nm]: 315 λ emission [nm]: 470

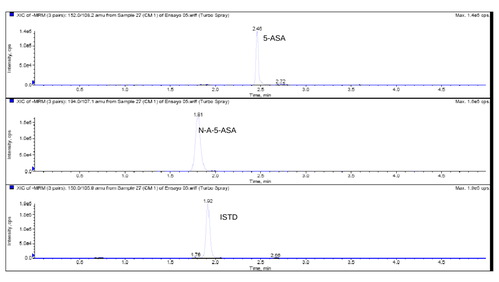
EXAMPLES OF CHROMATOGRAMS (LC-MS/MS METHOD)



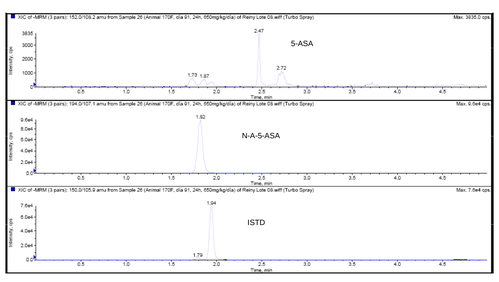
Blank plasma



LOQ (10 ng/mL)



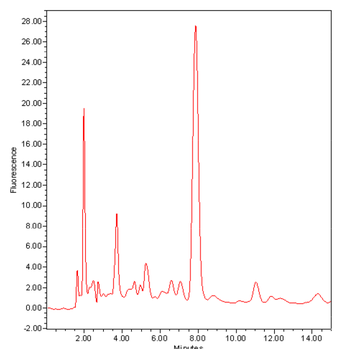
MQC(400 ng/mL)



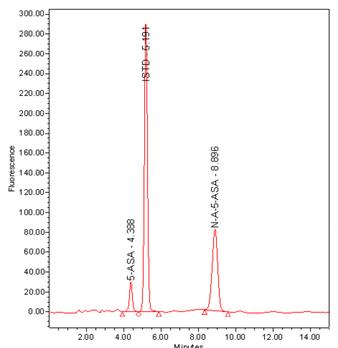
Incurred sample

Validated range: 10-1000 ng/mL

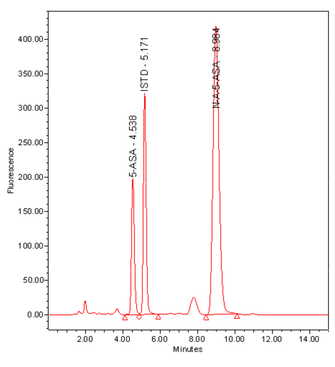
EXAMPLES OF CHROMATOGRAMS (HPLC-FLD METHOD)



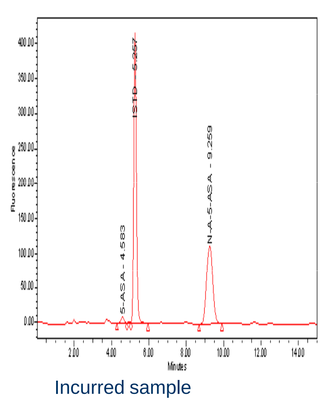
Blank plasma



LOQ (50 ng/mL)



MQC (400 ng/mL)



Incurred sample

Validated range: 50-1000 ng/mL

CONCLUSIONS

The new method has made possible to change the plasma extraction process by using a simple protein precipitation, reducing the time for each analysis one third.
And, besides that, the volume of plasma has been reduced from 0.3 mL to 0.1 mL.
Both methods have been fully validated according to FDA requirements with the aim of applying them to the analysis of plasma samples of different species from preclinical studies or clinical trials.