Evaluation of the Budesonide 22R and 22S Epimers Fragmentation in LC-MS/MS and its Impact in Quantitative Bioanalysis

Eugénie-Raphaëlle Bérubé , Sylvain Latour, Milton Furtado and Fabio Garofolo*

Algorithme Pharma Inc., Laval (Montreal), Quebec, CANADA

Algorithme Pharma
AN ALTASCIENCES COMPANY

OVERVIEW

- **Purpose**
  - Investigation of the different fragmentation of budesonide epimers and the impact on quantification, when using a co-eluting chromatographic method.

Method

- Budesonide racemate mixture reference standard was evaluated with UV detection under chromatographic conditions permitting the separation of the two epimers.
- By LC-MS/MS in both detection modes, fragmentation/ionization of the two budesonide epimers was evaluated and compared against the UV data.
- Pure solution of 22R and 22S budesonide were separately prepared throughout a concentration range and evaluated against a pure calibration curve of a reference standard containing a racemic mixture.

Results

- The reported concentration of budesonide is affected when chromatographic co-elution and different MRM transitions of these epimers are used.

INTRODUCTION

Budesonide is a potent nonhalogenated glucocorticoid consisting of a racemic mixture of the two epimers 22R and 22S. Depending on the mode of administration, the biodistribution of the two epimers may differ. When administrating intranasally, the volume of distribution of the 22R epimer is almost twice that of the 22S epimer, resulting in a different concentration of these two forms in plasma.

When employing chromatographic co-elution of the R and S epimers for budesonide quantification, it is important to ensure that the two epimers demonstrate similar fragmentation patterns; otherwise the concentration reported will be biased.

In the present work, we describe the impact of the budesonide epimers fragmentation differences on the quantification of samples with different R and S epimers ratio.

METHOD

Pure solutions of 22R and 22S budesonide (**Figure 1**) were separately prepared and evaluated against a pure calibration curve of a reference standard containing a mixture of budesonide. To compensate for system instability, the area ratio response was monitored with the addition of budesonide-D9 reference internal standard.

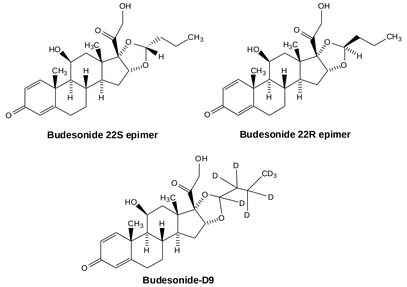


Figure 1: Structures of Budesonide Epimers and Internal Standard

CHROMATOGRAPHY:

The analysis was performed under reversed phase chromatography on an Agilent Technologies 1100 HPLC pumps and autosampler.

DETECTION:

Negative mode:

- The acetate adduct 489.2 m/z with product ions m/z 59.1 and 357.2 were monitored.
- AB SCIEX API3000.
- Mobile phase: 0.02% acetic acid with ACN.

Positive mode:

- The MRM 431.2/323.1 was monitored.
- AB SCIEX API5000.
- Mobile phase: 20mM Ammonium Bicarbonate pH 10 and ACN.

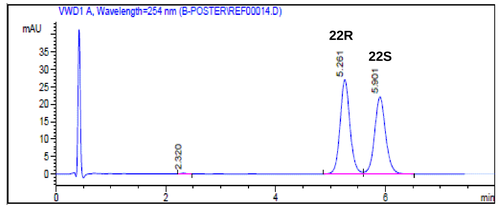
UV detection mode:

- Wavelength of 254 nm.
- Mobile phase: 0.02% acetic acid with ACN.

RESULTS

The reference standard mixture, using UV spectra showed similar proportions for the two budesonide forms. As demonstrated in **Figure 2**, the UV data confirms that the budesonide reference standard racemic mixture contains 52% of 22R epimer and 48% of 22S epimer. Accordingly, when performing an LC-MS method, both forms should demonstrate similar proportions as the UV analysis.

Figure 2. Representation of a UV Spectra of a Chromatographically Separated 22R and 22S Budesonide Racemic Reference Standard



Meas.RT	Main Peak Area	Main Peak Height
2.320	1.429	0.229
5.261	338.770	26.999
5.901	309.186	22.075

Quantitation results in a ratio of 52.3% of 22R epimer and 47.6% of 22S epimer.

Using LC-MS/MS detection, the best sensitivity for budesonide was achieved by electrospray in negative mode, when monitoring the acetate adduct 489.4 m/z. The loss of the acetate (SRM 489.4/59.1) gave similar intensity for the two epimers (ratio 50.7:49.3 R:S) but could not be used due to high background noise.

On the other hand, when monitoring the SRM m/z 489.2/357.2, a different ratio between the 22R and 22S was observed resulting in a proportion of 64.8%:35.2% (R:S). As demonstrated in **Figure 3**, all other SRM transitions in negative mode resulted in similar proportions.

However, as presented in **Figure 4**, the product ions obtained in positive mode had a ratio closer to the UV data. The selected product ion 323.1 m/z resulted in a proportion of 55.2% of 22R and 44.8% of 22S. The same ratio was also obtained for other SRM transition tested in this mode.

Figure 3: LC-MS/MS Chromatograms of 22R and 22S Budesonide Epimers Monitoring Different Fragment of the Acetate Adduct $[M\text{-}H\text{+}CH_3COOH]^-$ in Negative Mode Detection

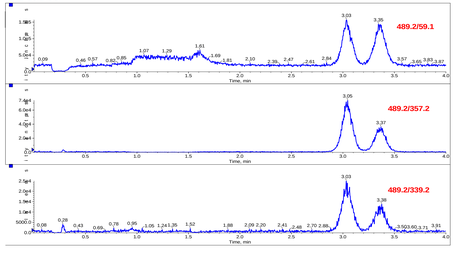
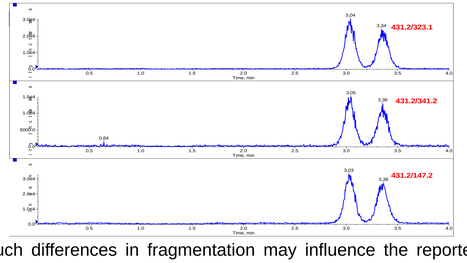


Figure 4: LC-MS/MS Chromatograms of 22R and 22S Budesonide Epimers Monitoring Different Fragment of the $[M+H]^+$ in Positive Mode



Such differences in fragmentation may influence the reported concentration if the R and S epimers chromatographically co-elute. To evaluate the impact on the quantification, each form of R and S budesonide was prepared separately at different concentrations and then compared against the calibration curve containing the mixture of both epimers.

Results for the 22R budesonide presented in **Table 1** showed a positive bias of 26% for the negative mode SRM 489.4/357.1 transition when compared to the positive mode using the SRM 431.2/323.1 transition.

For the epimer 22S, the impact is more important and resulted in a reported concentration lower than 35% when compared to the positive mode (**Table 2**). These results suggest that the different fragmentation of these two epimers in negative mode will affect the reported concentration if a co-eluting chromatographic method is used.

Table 1: 22R Budesonide Back-Calculated Concentration Against a Racemic Calibration Curve and the Variation of Different Fragment Monitored from the Positive Ion Mode Reported Data

Positive mode	Negative mode			
431.2/323.1	489.2/59.1		489.2/357.2	
Back calculated conc. (ng/mL)	Back calculated conc. (ng/mL)	% difference	Back calculated conc. (ng/mL)	% difference
46.14	38.72	-17.5	49.19	6.4
202.96	189.48	-6.9	242.98	17.9
228.21	226.96	-0.5	297.47	26.3
462.97	437.81	-5.6	574.03	21.4
386.88	367.94	-5.0	482.31	22.0
260.08	236.36	-9.6	308.55	17.0
198.85	180.54	-9.7	234.50	16.5
151.76	144.13	-5.2	185.89	20.2
127.82	114.63	-10.9	148.85	15.2
89.65	86.02	-4.1	111.29	21.5
68.93	62.15	-10.3	81.57	16.8
55.87	46.57	-18.2	60.41	7.8
34.38	33.27	-3.3	42.85	21.9
25.33	25.60	1.1	32.24	24.0
18.42	18.26	-0.9	23.38	23.7
11.79	10.46	-11.9	13.06	10.3
7.32	6.35	-14.2	8.24	11.7

Table 2: 22S Budesonide Back-Calculated Concentration Against a Racemic Calibration Curve and the Variation of Different Fragment Monitored from the Positive Ion Mode Reported Data

Positive mode	Negative mode			
431.2/323.1	489.2/59.1		489.2/357.2	
Back calculated conc. (ng/mL)	Back calculated conc. (ng/mL)	% difference	Back calculated conc. (ng/mL)	% difference
46.58	51.67	10.4	34.80	-28.9
230.65	251.68	8.7	170.35	-30.1
270.79	305.34	12.0	209.61	-25.5
513.92	563.43	9.2	391.59	-27.0
461.90	488.12	5.5	338.19	-30.9
297.0	328.24	10.0	223.66	-28.2
226.49	247.24	8.8	167.42	-30.0
175.67	190.44	8.1	129.03	-30.6
145.63	158.86	8.7	107.06	-30.5
114.92	123.81	7.5	82.66	-32.7
81.21	84.80	4.3	56.15	-36.5
59.70	65.51	9.3	42.88	-32.8
43.05	45.13	4.7	30.18	-35.1
34.39	36.56	6.1	24.10	-35.2
23.02	24.83	7.6	16.12	-35.2
13.11	14.71	11.5	9.73	-29.6
8.56	9.48	10.2	6.47	-27.8

CONCLUSION

In conclusion, the ratio of the two epimers may differ in subject samples throughout the pharmacokinetic profile and cause a bias on the calculated concentration if the same fragmentation/ionisation pattern is not observed.

Accordingly, a UV comparison should be done and compared to an LC-MS method to determine if the epimers can be merged or if they must be chromatographically separated.

* CORRESPONDING AUTHOR