QUANTITATION OF BASIC COMPOUNDS IN HIGH pH MOBILE PHASES BY LC/MS/MS WITH ELECTROSPRAY IN PIM

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Introduction

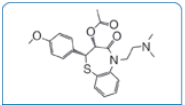
There is considerable interest in conducting reversed-phase HPLC separations at pH well above the pK_a values of basic compounds[1-3]. When the pH of the mobile phase is two pH units higher than the pK_a values of basic analytes, the uncharged species are better retained on reversed-phase stationary phases, resulting in increased retention times of polar basic analytes without using ion-pairing reagents; more reproducible retention; superior peak shapes; alternative column selectivity[4], and also elution in a more favorable mobile phase, having a higher organic content which is beneficial for LC/MS detection.

One possible drawback could be a severe decrease in sensitivity with mass-spectrometric detection, under conditions that suppress analyte ionization in solution. As the ionization state of analyte molecules depends on the pH of the mobile phase, it is expected that the ionization efficiency in LC/MS with electrospray (ESI) in positive ion mode (PIM) be drastically lowered in high pH mobile phases. Thus the LC/MS analysis of basic drug compounds in mobile phases of high pH (>pK_a+2) could be compromised if gas-phase ionization yields in ESI+ were closely linked to acid-base equilibria in solution. Nevertheless, several workers have reported the successful detection of particular basic compounds in ESI$^+$ when using high pH buffers in the mobile phase[5-11].

Introduction (cont)

In our previous work[5], we compared the ESI^+ responses of various groups of basic drugs within a wide range of polarity (logP 0.09~7.6) and pK_a values (8~10), in low and high pH mobile phases. Analyte signal intensities observed in 0.1% formic acid with acetonitrile were compared to intensities observed in 10mM ammonium bicarbonate buffers at different pH (7.8-11), with acetonitrile, as mobile phase components. Contrary to common expectations, high pH mobile phases do NOT suppress the ionization of basic compounds in ESI^+; positive ions are formed abundantly, and analyte responses are comparable, or most often better in high pH compared to acidic mobile phases. In this poster we further investigated the effectiveness of using high pH mobile phases for the quantitation of basic compounds in ESI^+ LC/MS/MS by comparing limits of detection (LOD), limits of quantitation (LOQ), linearity ranges, precision, and accuracy observed in high and low pH mobile phases.

Molecular Structures of Basic Analytes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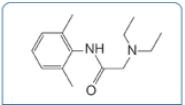
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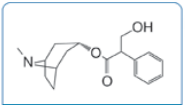
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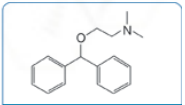
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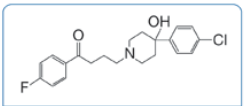
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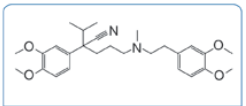
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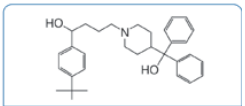
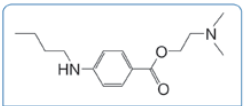

Terfenadine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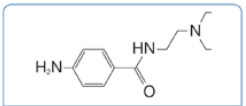
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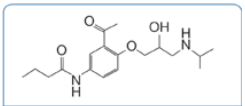
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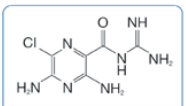
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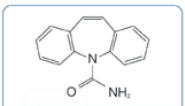
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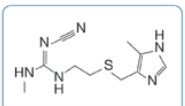
Cimetidine

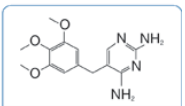
Trimethoprim

Table 1. Analyte Characteristics

Compound	MS/MS Transition	pK_a, pK_{a2}	LogP
Diltiazem	415 → 178	8.91	2.70
Lidocaine	235 → 86	8.01	2.44
Atropine	290 → 124	9.43	1.83
Diphenhydramine	256 → 167	8.98	3.27
Haloperidol	376 → 165	8.66	4.30
Acebutolol	337 → 116	9.20	1.71
Cimetidine	253 → 159	6.80	0.40
Terfenadine	472 → 436	9.57	7.62
Carbamazepine	237 → 194	–	2.45
Tetracaine	265 → 176	8.20	3.51
Procainamide	236 → 163	9.32	0.88
Amiloride	230 → 171	8.70, 9.30	0.09
Verapamil	456 → 165	8.92	3.79
Trimethoprim	291 → 230	7.12	0.91

Experimental Conditions

Instrumentation:	
HPLC System:	HP 1100 series (*www.agilent.com*)
Pump:	G1312A (Binary Pump)
Autosampler:	G1329A ALS
MS Detector:	API 3000 LC/MS/MS (*www.appliedbiosystems.com*), with ESI (TurboIonSpray®) operated in PIM
Mobile Phase:	
Low pH Mobile Phase: High pH Mobile Phase:	A: 0.1% Formic Acid in Water; B: 0.1% Formic Acid in Acetonitrile A: 10mM Ammonium Bicarbonate, pH=7.8, 9 and 10; B: Acetonitrile
Gradient:	A/B (90:10) to (10:90) in 10min, hold for 2min; re-equilibrate for 4min
Flow Rate:	0.6mL/min
Column:	Gemini™ 5µm C18, 150 x 3.0mm ID
Concentration Levels:	0.05 - 200ng/mL
Injection Volume:	5µL

Figure 1. LC/MS/MS Responses for Basic Compounds in pH=2.7 and pH=10.0 Mobile Phases

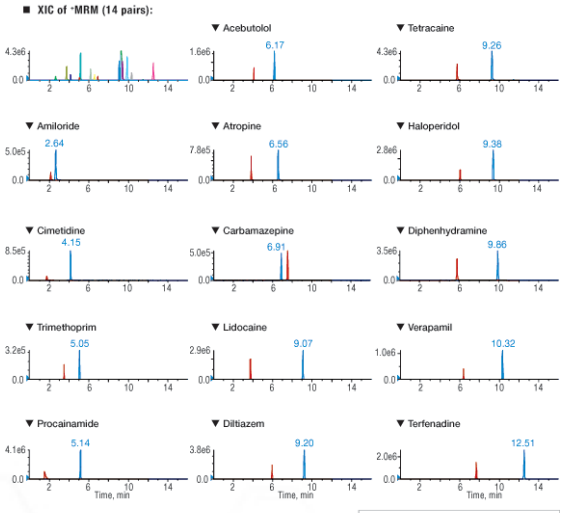

Figure 2. Comparison of LC/MS/MS Responses in Acidic and Basic Mobile Phases

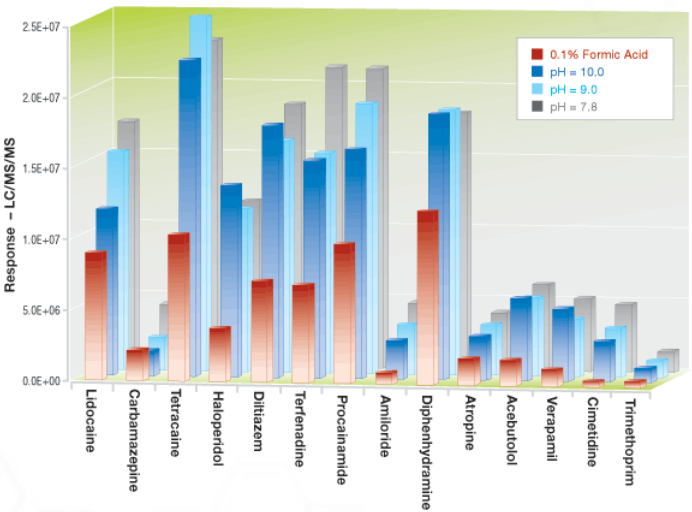

Figure 3. Reversal in Elution Order in Low pH and High pH Mobile Phases

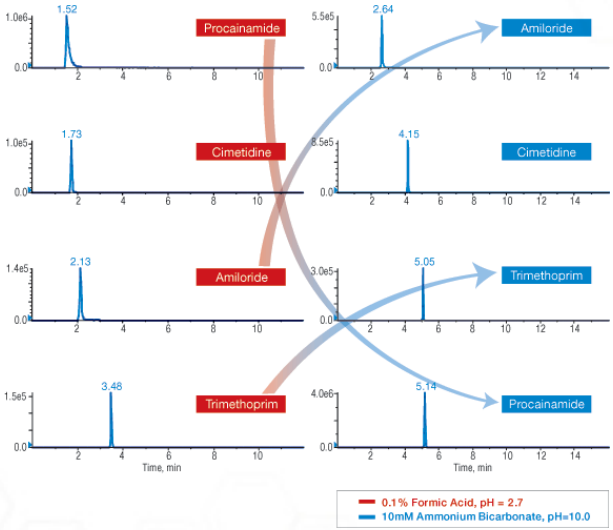

Figure 4. LC/MS/MS Responses of Basic Compounds at the 50pg/mL Level (pH=10.0)

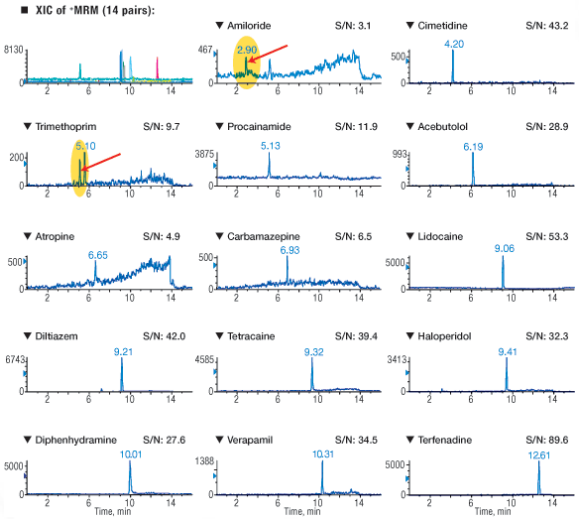

Figure 5. LC/MS/MS Responses of Basic Compounds at the 50pg/mL Level (pH=2.7)

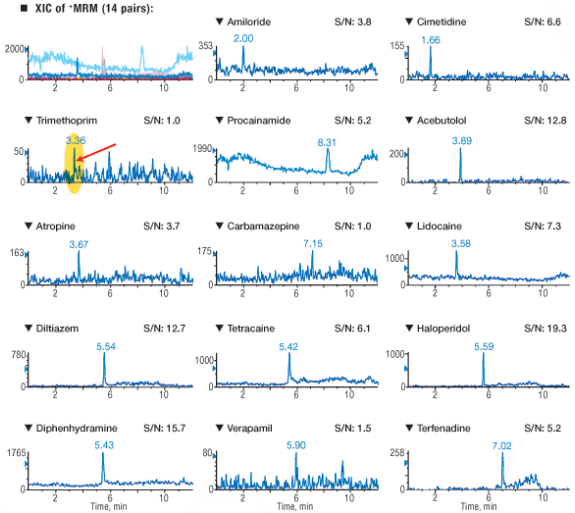

Figure 6. S/N in LC/MS/MS in Acidic and Basic Mobile Phases at the 50pg/mL Level

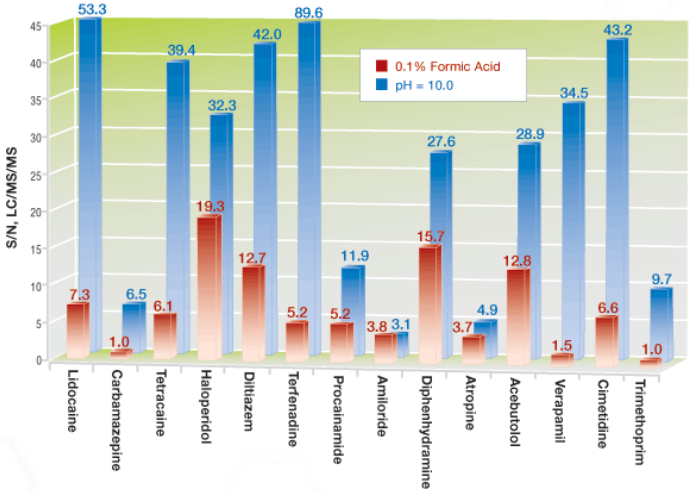

Table 2. Quantitation of Basic Compounds - LOQ and Linearity

	LOQ (S/N=10, fg on column)		**Linearity** (pH=10) *1		
Compound Name	**Acidic pH**	**pH = 10**	**Dynamic Range**	**R^2 *2, *3**	**Range (ng/mL)**
Lidocaine	> 500	> 50	2 X 10^3	0.9993	0.05 - 100
Carbamazepine	> 1750	> 1250	1 X 10^3	0.9999	0.10 - 100
Tetracaine	> 500	> 65	2 X 10^3	0.9998	0.05 - 100
Haloperidol	> 125	> 85	2 X 10^3	0.9997	0.05 - 100
Diltiazem	> 250	> 250	2 X 10^3	0.9981	0.05 - 100
Terfenadine	> 500	> 250	2 X 10^3	0.9984	0.05 - 100
Procainamide	> 500	> 250	2 X 10^3	0.9993	0.05 - 100
Amiloride	> 1250	> 750	1 X 10^3	1.0000	0.10 - 100
Diphenhydramine	> 250	> 100	2 X 10^3	0.9988	0.05 - 100
Atropine	> 500	> 500	1 X 10^3	0.9996	0.10 - 100
Acebutolol	> 250	> 100	2 X 10^3	0.9996	0.05 - 100
Verapamil	> 1250	> 85	2 X 10^3	0.9992	0.05 - 100
Cimetidine	> 500	> 65	2 X 10^3	0.9996	0.05 - 100
Trimethoprim	> 1750	> 500	1 X 10^3	0.9975	0.05 - 100

**1 Intra-assay (n=6); *2 Duplicated injections at each level; *3 N = 5 or 6 pts*

Table 3. Quantitation of Basic Compounds - Precision and Accuracy

	Precision, RSD% (pH=10) *1				Accuracy (%) (pH=10) *1			
Compound Name	0.05ng/L	0.25ng/L	1.00ng/mL	100ng/mL	0.05ng/mL	0.25ng/mL	1.00ng/mL	100ng/mL
Lidocaine	12.21	11.90	4.70	3.84	107	100	96	100
Carbamazepine	9.92 *2	7.23	8.19	2.72	72	122	100	100
Tetracaine	12.99	3.93	4.70	4.03	115	89	99	100
Haloperidol	9.49	3.07	5.62	4.37	135	90	103	100
Diltiazem	14.67	6.70	6.35	5.60	94	84	108	100
Terfenadine	6.09	5.75	1.47	7.26	161	80	114	100
Procainamide	11.52	8.03	6.63	5.19	86	81	100	100
Amiloride	–	2.93	11.10	4.92	–	101	93	100
Diphenhydramine	11.41	7.12	4.34	8.38	102	82	111	100
Atropine	13.03 *2	6.07	7.38	5.94	76	69	100	100
Acebutolol	11.47	6.43	10.18	5.06	94	84	93	100
Verapamil	13.21	8.62	11.41	11.58	138	85	104	100
Cimetidine	11.70	9.78	7.22	5.72	93	111	97	100
Trimethoprim	11.03 *2	5.79	6.98	6.87	90	102	96	100

*1 Intra-assay (n=6); *2 S/N < 10

Results and Discussion

Influence of Mobile Phases and pH Buffer

In high pH mobile phase, the reversed-phase elution of basic compounds in uncharged state, both polar and non-polar, results in significantly longer retention times, sharper peak shapes **(Figure 1 and 2)**, and often changed elution order (=alternative column selectivity) **(Figure 3)**. All these changes result in a significant improvement in column resolving power, and also elution in a more favorable mobile phase - having a higher organic content (high efficiency of desolvation) which is beneficial in LC/MS detection **(Figure 1 and 2)**.

Limit of Quantitation and Response Linearity

The limit of quantitation (LOQ) is considered to be the minimum analyte quantity on-column giving a S/N of 10. Most basic compounds included in this study were successfully quantified at the level of 1.25pg on-column, or better, some at levels as low as 50fg **(Table 2)**. A comparison of S/N ratios at very low concentration levels (50pg/mL) reveals that most basic compounds included in this study can be detected with better sensitivity in high pH mobile phases **(Figure 4, 5, 6, and Table 2)** compared to acidic conditions. Only Carbamazepine (LOQ>1250fg), and Amiloride (pK_{a1} 8.7; pK_{a2} 9.3; LOQ>750fg), were detected with lower S/N in high pH mobile phase. Response linearity was studied in the concentration range 0.05-100ng/mL for all basic compounds. The results **(Table 2)** demonstrate good linearity in all cases with R^2 values above 0.997 in a wide dynamic range (>103).

Precision and Accuracy

Method precision was determined by replicate analyses (n = 6) at four concentration levels: 0.05ng/mL, 0.25ng/mL, 1.00ng/mL and 100ng/mL. **Table 3** shows RSD < 15% at low concentration level, and <12% at the 100ng/mL level. Method accuracy was evaluated by comparing the mean value of six replicate analysis with the expected concentration value, at different levels. The analyzed concentrations were calculated from the equation **$y = mx \pm b$** as determined by linear regression of results obtained on standard calibrators. Accuracy was better than 80% for most analytes when quantified at their respective LOQ level, and 100% at the 100ng/mL level **(Table 3)**.

Conclusions

- The effectiveness of using high pH mobile phases for the quantitation of basic drugs covering a wide range of polarity (logP–0.4~7.6) and pK_a values (6.5~10) in ESI^+ LC/MS/MS was evaluated by comparing limits of detection (LOD) and quantitation (LOQ), linearity ranges, and accuracy observed in low and high pH mobile phases.
- The successful quantitation of these basic compounds by ESI+ LC/MS/MS in a mobile phase containing a high pH buffer was achieved.
- The solution–phase ionization of basic analytes at weak acid or low pH buffer condition for ESI is necessary for enhancing MS response but not mandatory. The most basic compounds included in this study can be detected with better sensitivity in high pH mobile phases of ammonium bicarbonate-ammonium hydroxide buffer compared to low pH mobile phase of formic acid.
- The results demonstrated the strong feasibility of performing basic compound analysis in high pH mobile phases by ESI^+ LC/MS/MS with higher sensitivity, good linearity, precision and accuracy, when using the high performance **Gemini™ 5µm C18** HPLC column.

References

1. Alfonso Espada, Alfonso Rivera-Sagredo, *J. Chromatogr A*, 2003, 987, 211-220
2. M.W.Dong, G.D. Miller, R.K. Paul, *J. Chromatogr A*, 2003, 987, 283-290
3. Lawrence Loo and Tivadar Farkas poster presentation at *Pittcon 2005*, Orlando, Fl
4. C. Mallet, Z. Lu, J. Mazzeo and U. Neue, *Rapid Commun. Mass Spectrom.*, 2002, 16: 805-813
5. Liming Peng and Tivadar Farkas, poster presentation at *HPLC 2005*, Stockholm, Sweden
6. S. Zhou and Kelsey Cook, *J. Am. Soc. Mass Spectrom.*, 2000, 11, 961-966
7. Y. Cheng, Z. Lu and U. Neue, *Rapid Commun. Mass Spectrom.*, 2001, 15: 141-151
8. F. Kuhlmann, A. Apffel, S. Fischer, G. Goldberg and P. Goodley, *J. Am. Soc. Mass Spectrom.*, 1995, 6, 1221-1225
9. G. Wang and R Cole, *Organic Mass Spectrometry*, 1994, 29, 419-427
10. C. Mallet, Z. Lu and J. Mazzeo, *Rapid Commun. Mass Spectrom.*, 2004, 18: 49-58
11. M. Kelly, M. Vestling and C. Fenselau, *Organic Mass Spectrometry*, 1992, 27, 1143-1147