

Analysis of Potential Genotoxic Arylamine and Aminopyridine Impurities in Active Pharmaceutical Ingredients

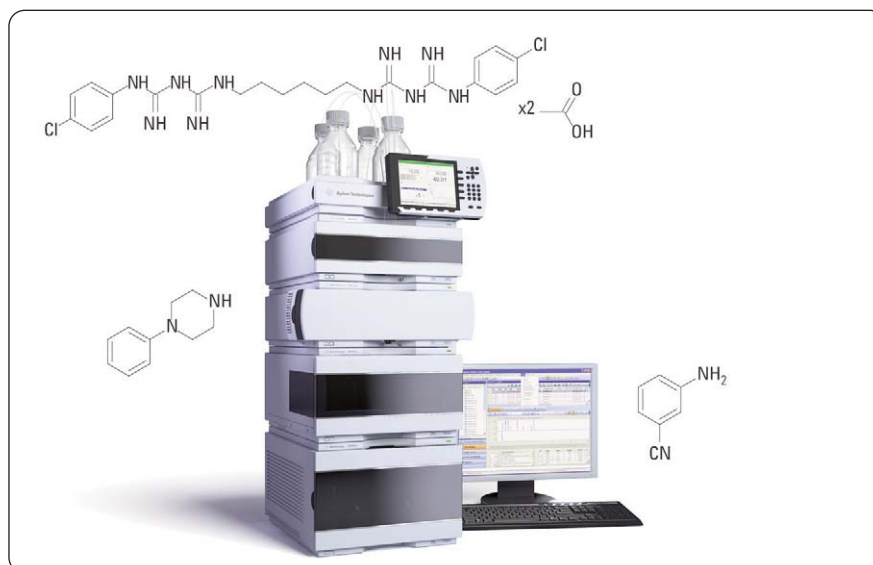
Agilent 1290 Infinity LC system and Agilent 6460A Triple Quadrupole MS system for method development and fast analysis

Application Note

Pharmaceutical

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Abstract

This Application Note illustrates the possibilities of the Agilent 1290 Infinity LC system and the Agilent 6460A Triple Quadrupole Mass Spectrometer for quantitative analysis of potential genotoxic impurities (PGIs) in active pharmaceutical ingredients (APIs). A generic approach was used to determine arylamine and aminopyridine PGIs in selected APIs. The influence of column length and mobile phase composition on the recovery of these impurities in real samples were investigated. High resolution UHPLC using a 150 mm RRHD column packed with 1.8 μm particles resulted in the best overall performance. In cases of good separation between API and the target solutes, analysis speed and sample throughput could be increased using a 50 mm RRHD column.

Detection limits were below 20 ppb (ng/g, relative to the API) using MS/MS and below 100 ppb (ng/g, relative to the API) with DAD for 2,6-dichloroaniline, which could not be detected with ESI-MS at trace levels.



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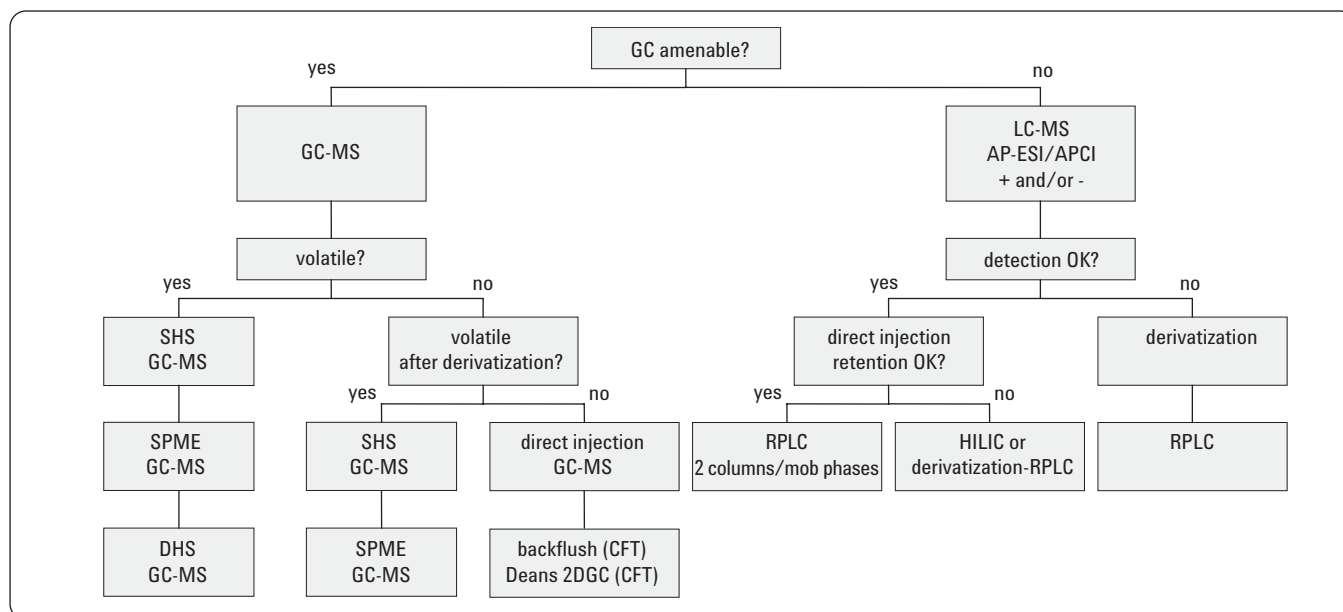


Figure 1
Method selection flow chart for PGI analysis in APIs.³

Introduction

Impurities in pharmaceutical ingredients containing a structural alert functionality are called potential genotoxic impurities (PGIs). These impurities result from degradation of the active pharmaceutical ingredient (API), excipients, or residues from the production of the substance. PGIs have received increased attention recently and a threshold of toxicological concern (TTC) has been issued.¹ A TTC value of 1.5 µg/day intake of a genotoxic impurity is considered to be an acceptable risk or a risk in which the possibility of significant carcinogenicity is very low. The Committee for Medicinal Products (CHMP) defines an acceptable risk as an additional cancer risk of <1 in 100,000 based on a lifetime exposure to the genotoxic impurity.² Consequently, analytical methods should detect these impurities at ppm levels in APIs. Recently an overview of methods for various classes of PGIs was published³ and a method selection chart based on some straightforward questions was presented (Figure 1).

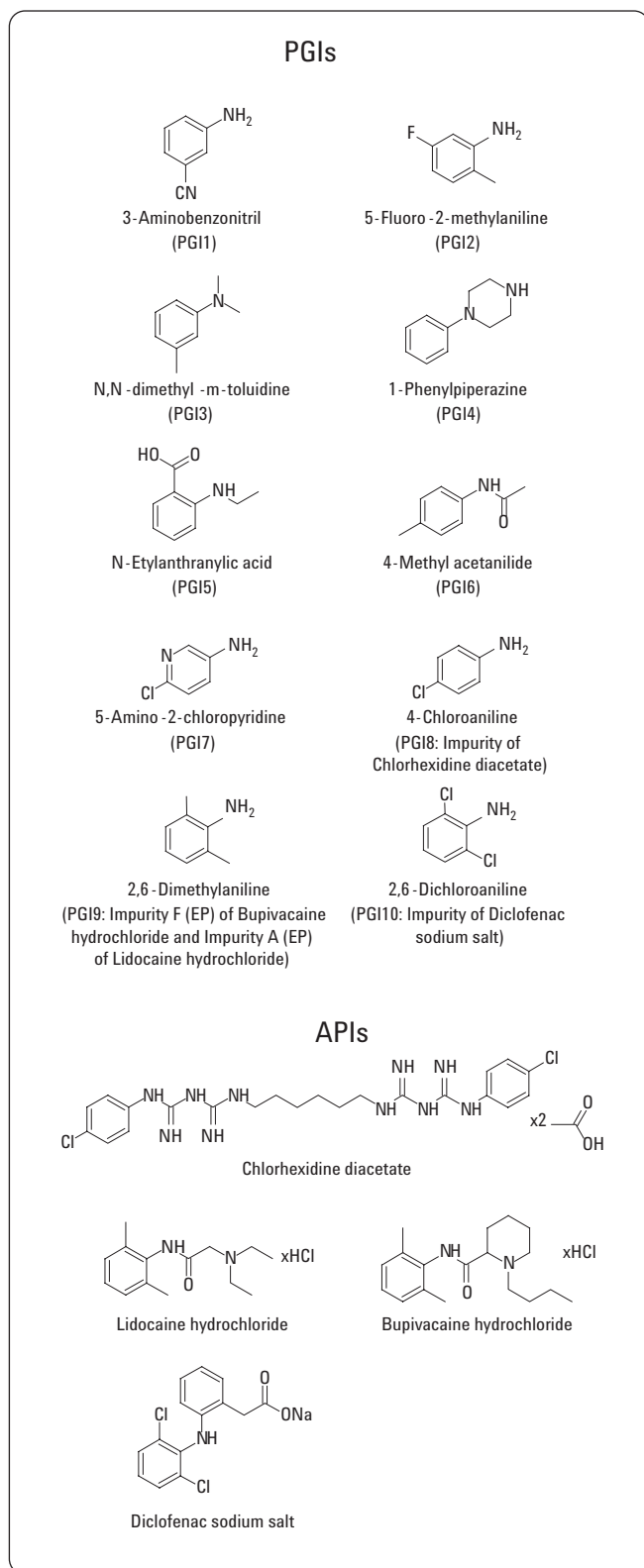
Arylamines and aminopyridines are building blocks of APIs and these PGIs can potentially be present at trace levels in various pharmaceuticals. Their analysis at the sub-ppm level in APIs is challenging and requires state-of-the-art instrumentation providing the necessary sensitivity to detect trace concentrations of the PGI, and the selectivity to reduce matrix interference. Recently we described the analysis of this class of compounds at trace levels using a single quadrupole LC-

MSD system.⁴ The use of triple quadrupole MS can further enhance selectivity and sensitivity. The quantification of a PGI (EP Impurity D) in atenolol using the Agilent 1200 Series Rapid Resolution LC (RRLC) combined with an Agilent 6410B Triple Quadrupole Mass Spectrometer demonstrated the performance of this approach.⁵

To further enhance the sensitivity and speed of analysis, an Agilent 1290 Infinity UHPLC system combined with an Agilent 6410B Triple Quadrupole LC/MS system was applied to analyze ten arylamines and aminopyridines in four different APIs. The structures of the investigated substances are shown in Figure 2. Some of the PGIs are known impurities of the selected APIs. To test the recovery in real samples a highly concentrated solution of the API (with or without spiking) was analyzed in MRM mode. One of the PGIs (2,6-dichloroaniline) gave low response in MS (using ESI ionization) and was detected using DAD.

Agilent ZORBAX RRHD columns packed with 1.8 µm particles were used in this study and the power of the Agilent 1290 Infinity LC system allowed the use of different column lengths.

Selection between methanol and acetonitrile was used to optimize the chromatographic selectivity to avoid or reduce matrix interferences.



Experimental

Instrumentation

An Agilent 1290 Infinity LC system equipped with an Agilent 1290 Infinity Diode Array Detector and an Agilent 6460A Triple Quadrupole LC/MS system with Agilent Jet Stream technology were used. The Agilent 1290 Infinity LC system was configured as follows:

G4220A	Agilent 1290 Infinity Binary Pump with integrated vacuum degasser
G4226A	Agilent 1290 Infinity Autosampler
G1316C	Agilent 1290 Infinity Thermostatted Column Compartment
G4212A	Agilent 1290 Infinity Diode Array Detector

Method parameters

Column	Agilent ZORBAX Eclipse Plus C18 RRHD, 50, 100 or 150 mm l × 2.1 mm id, 1.8 μm
Mobile phase	A=0.05% formic acid in water B=methanol or acetonitrile
Flow rate	0.5 mL/min

Gradient	Column Length			
	50 mm	100 mm	150 mm	
B=Methanol	0-0.25 min	0-0.5 min	0-0.75 min	10% B
	0.25-2.5 min	0.5-5 min	0.75-7.5 min	10-100% B
	2.5-3 min	5-6 min	7.5-9 min	100% B
	3-4 min	6-7.5 min	9-11.2 min	10% B
B=Acetonitrile	0-0.25 min	0-0.5 min	0-0.75 min	5% B
	0.25-2.5 min	0.5-5 min	0.75-7.5 min	5-95% B
	2.5-3 min	5-6 min	7.5-9 min	95% B
	3-4 min	6-7.5 min	9-11.2 min	5% B

Temperature	B=methanol: 45 °C B=acetonitrile: 40 °C
Injection	MS: 1 μL, with needle wash (flushport, 5 s, water/methanol 1/1) DAD: 5 μL, with needle wash (flushport, 5 s, water/methanol 1/1)

Detection

DAD (PGI10)	
Signals	Signal 238/5 nm; Reference 420/50 nm Signal 296/10 nm; Reference 450/50 nm
Cell	Standard 10 mm flow cell.
Slit	4 nm
Peakwidth	>0.012 min (20 Hz)

Figure 2
Structures of PGIs and APIs under investigation.

MS/MS (PGI1-9)

Ionization	Electrospray with Jet Stream technology positive ionization	
Jet Stream parameters	Drying gas temperature	330 °C
	Drying gas flow	8 L/min
	Nebulizer pressure	35 psig
	Sheath gas temperature	340 °C
	Sheath gas flow	10 L/min
	Capillary voltage	4000 V
	Nozzle voltage	500 V
Acquisition	50 mm column	0.6 to 2.6 min Time filtering Off
	100 mm column	1.2 to 5 min Time filtering 0.02 min
	150 mm column	1.8 to 7.5 min Time filtering 0.02 min
	MRM settings	Table 1
	Delta EMV	50

PGI Name	Precursor ion (m/z)	Product ion (m/z)	Fragmentor (V)	Collision energy (V)
1 3-aminobenzonitril	Q 119.1	92.0	110	15
	q 119.1	102.0	110	21
2 5-fluoro-2-methylaniline	Q 126.1	111.0	110	17
	q 126.1	109.0	110	17
3 N,N-dimethyl-m-toluidine	Q 136.1	121.0	110	13
	q 136.1	120.0	110	25
4 1-phenyl-piperazine	Q 163.1	120.0	120	18
	q 163.1	118.1	120	25
5 N-ethylanthranylic acid	Q 166.1	130.0	90	17
	q 166.1	148.0	90	5
6 4-methyl acetanilide	Q 150.1	108.0	100	13
	q 150.1	93.0	100	25
7 5-amino-2-chloropyridine	Q 129.1	93.0	120	18
	q 129.1	66.0	120	25
8 4-chloroaniline	Q 128.1	93.0	120	17
	q 128.1	75.0	120	30
9 2,6-dimethylaniline	Q 122.1	105.1	110	16
	q 122.1	77.0	110	25
10 2,6-dichloroaniline	DAD (no MS response)			

Table 1
Data acquisition parameters for the compounds under investigation. Q = transition for quantification, q = transition for confirmation.

Chemicals

All PGIs and APIs were from Sigma-Aldrich.

- Bupivacaine hydrochloride, >99%, 5g
- Chlorhexidine, 98%, 25g
- Lidocaine hydrochloride monohydrate, solid, 15g
- Diclofenac sodium salt, 10g

Standard solutions

Individual stock solutions (1-3 mg/mL) of the PGIs were prepared in acetonitrile. The solutions were mixed and diluted in acetonitrile to obtain a 100 µg/mL mixture which was further used to prepare the calibration and standard solutions.

Sample preparation

The following APIs were selected:

- Bupivacaine hydrochloride (Purity min. 99%)
- Lidocaine hydrochloride (Purity min. 99%)
- Chlorhexidine diacetate (Purity min. 97.5%)
- Diclofenac sodium salt (Purity min. 98%)

The sample preparation procedure is described below. Some APIs were not completely dissolved after ultrasonic agitation. The solubility of the PGI in the extraction solvent is excellent and spiking experiments have demonstrated that the recovery is higher than 70%⁴.

- Weigh 120 mg sample into a 1.5-mL Eppendorf tube
- Add spiking solution if necessary
- Add 1.2 mL acetonitrile (API is at 10%)
- Vortex, 30 s
- Ultrasonic batch, 5 min
- Vortex, 30 s
- Centrifuge at 13,000 rpm, 2 min
- Filter solution through a syringe filter (0.2 µm pore size, regenerated cellulose, Agilent p/n 5061-3366)

Results and discussion

Method optimization

Six generic methods were developed using three column lengths (5, 10 and 15 cm) and two organic modifiers (methanol and acetonitrile). Mobile phase A was always 0.05% formic acid in water. The MS conditions were constant for all methods. The LC-MS/MS MRM conditions were selected using concentrated solutions (10 ppm) of the individual target solutes. The Agilent MassHunter Optimizer Software was applied to automatically determine the product ion, the optimum fragmentor voltage, and the collision energy for each PGI.

Since the API sample solution is very concentrated (10% API) and the sample solvent is 100% acetonitrile, small injection volumes are mandatory to avoid peak distortion and peak broadening due to the strong sample solvent. In addition, the peak width of the main compound should be as narrow as possible to maximize the separation between the API and PGI and to eliminate or reduce interferences in UV detection and/or MS ionization. For this reason, a 1- μ L injection volume was chosen.

Extracted ion chromatograms, obtained for the analysis of a 10 μ g/ μ L standard solution (corresponding to 0.1 ppm in API for a 100 mg/mL API solution), are shown in Figure 3. This analysis was performed on a 100 mm \times 2.1 mm column packed with 1.8 μ m Agilent ZORBAX Eclipse Plus C18 RRHD material. A formic acid (0.05%) in water/acetonitrile gradient was used.

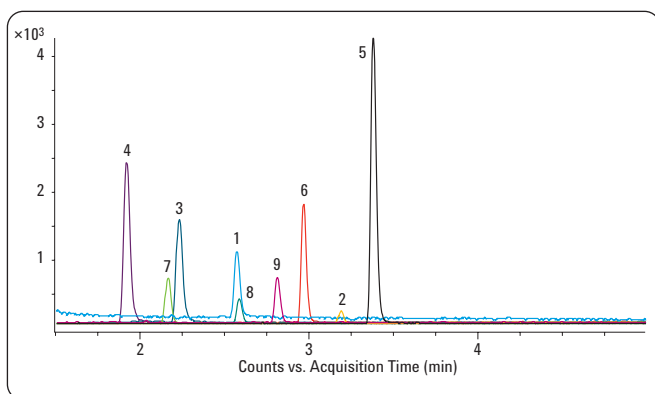


Figure 3
Extracted Ion Chromatograms from MRM of a 10 μ g/ μ L standard solution. Column: 100 mm, modifier: acetonitrile.

PGIs 1 to 9 can easily be detected. Using a 1 μ L injection volume, the detection limits for standard solutions were below 2 ng/mL using the Jet Stream ESI ionization source and operating in MRM mode.

One of the investigated PGIs (PGI10, 2,6-dichloroaniline) gives low response in electrospray ionization MS and was detected by DAD only. The UV spectrum of this compound showed two maxima, at respectively 238 nm and at 296 nm, and both wavelengths were evaluated. Because of the reduced sensitivity compared to the triple quadrupole detector, a larger sample volume was injected. Since PGI10 is the most retained impurity in the set of selected compounds a larger injection volume (5 μ L) was investigated. A comparison of a 1 and 5 μ L injections of 10 ng/ μ L and 2 ng/ μ L standard solutions is shown in Figure 4. While severe bandbroadening and peak distortion are observed for the early eluting com-

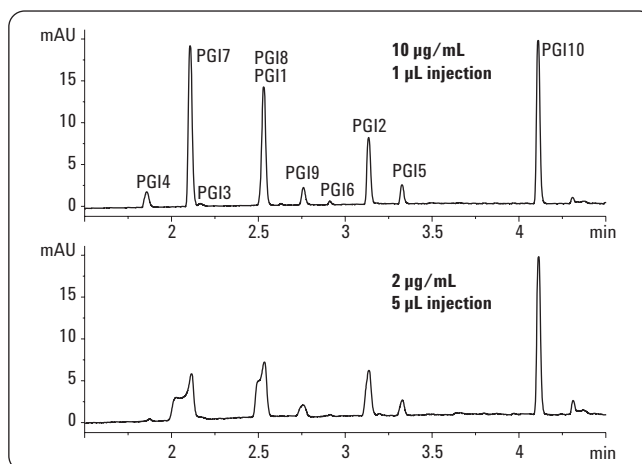


Figure 4
Chromatograms of standard solutions. Column: 100 mm, modifier: acetonitrile, detection: DAD 296 nm.

pounds, the retained PGI10 can be measured without loss of efficiency or sensitivity using the 5- μ L injection. For PGI10, the detection limit for standard solutions was below 10 ng/mL using DAD detection (5 μ L injection).

The six methods were validated. Linearity was measured for concentrations between 0.002 μ g/mL and 1 μ g/mL (MS/MS) and between 0.01 μ g/mL and 2 μ g/mL for DAD. Repeatability (n=5) was determined at 0.1 μ g/mL for MS/MS and at 0.2 μ g/mL for DAD. Limits of detection were calculated from the S/N measured at 0.002 μ g/mL (MS/MS) and at 0.01 μ g/mL (DAD). A summary of the method validation data is given in Table 2.

Linearity ¹	PGI1	PGI2	PGI3	PGI4	PGI5	PGI6	PGI7	PGI8	PGI9	PGI10 238 nm	PGI10 296 nm
ACN, 50 mm	0.9996	0.9997	0.9994	0.9986	0.9997	0.9999	0.9989	0.9997	0.9997	0.9999	0.9999
ACN, 100 mm	0.9990	0.9999	0.9993	0.9905	0.9978	0.9997	0.9999	0.9996	0.9998	0.9999	0.9999
ACN, 150 mm	0.9989	0.9980	0.9989	0.9991	0.9999	0.9994	0.9996	0.9993	0.9989	0.9999	0.9997
MeOH, 50 mm	0.9999	0.9995	0.9997	0.9977	0.9995	0.9998	0.9999	0.9996	0.9999	0.9999	0.9999
MeOH, 100 mm	0.9994	0.9998	0.9999	0.9989	0.9999	0.9998	0.9996	0.9998	0.9992	0.9999	0.9999
MeOH, 150 mm	0.9998	0.9993	0.9999	0.9889	0.9991	0.9999	0.9996	0.9998	0.9998	0.9999	0.9999
Repeatability ²	PGI1	PGI2	PGI3	PGI4	PGI5	PGI6	PGI7	PGI8	PGI9	PGI10 238 nm	PGI10 296 nm
ACN, 50 mm	1.86	2.26	0.97	1.79	1.62	1.98	0.29	1.34	1.49	3.82	1.14
ACN, 100 mm	1.68	1.45	0.36	1.65	2.38	2.23	2.03	1.86	1.25	1.26	2.70
ACN, 150 mm	1.30	1.48	0.75	1.68	0.94	0.54	1.39	1.84	1.22	1.15	3.68
MeOH, 50 mm	1.70	1.81	0.72	1.50	0.91	1.33	0.38	1.94	1.08	4.57	4.83
MeOH, 100 mm	1.76	2.29	1.07	1.04	0.61	1.15	1.02	0.55	1.10	3.19	0.90
MeOH, 150 mm	1.60	2.16	0.93	1.39	1.00	1.43	1.81	0.68	1.69	3.21	5.75
Sensitivity	PGI1	PGI2	PGI3	PGI4	PGI5	PGI6	PGI7	PGI8	PGI9	PGI10 238 nm	PGI10 296 nm
S/N ³	13.4	4.0	10.1	46.0	100.9	25.0	30.2	3.6	6.6	8.2	6.1
LOD (ng/ml)	0.5	1.5	0.6	0.1	0.1	0.2	0.2	1.7	0.9	3.7	4.9

- MS: 0.002-1 µg/mL, 1 µL injection, 1 injection/concentration
DAD: 0.01-2 µg/mL, 5 µL injection, 1 injection/concentration
- MS: 0.1 µg/mL, 1 µL injection, 5 consecutive injections
DAD: 0.2 µg/mL, 5 µL injection, 5 consecutive injections
- Signal-to-noise measured at 0.002 µg/mL (MS) and 0.01 µg/mL (DAD). LOD in ng/mL calculated for S/N=3.
Analyses performed with acetonitrile on 100-mm column.

Table 2
Method performance results.

All methods performed very well with r^2 values above 0.995, except for the first eluting PGI4 ($r^2 = 0.990$ with 100 mm column and acetonitrile). RSDs were typically below 3% using the MRM mode.

Influence of the column length

The major bottleneck in the determination of PGI in an API is possible coelution of the target solute with the API (or another impurity at high relative concentration), especially when a generic method is used. Chromatographic resolution is a key requirement for this type of analysis. Increasing the efficiency and peak capacity is a valuable tool in improving the separation and reducing interference of the main product (or a high level impurity) on the recovery and accurate quantification of the impurities. A straightforward way to increase the efficiency is to increase the column length.

Three columns of different length, but same ID and packing size were compared. In order to maintain the selectivity in each case, gradient times were changed accordingly (scaled methods). The influence of the column length on accuracy is illustrated in Figure 5, where the overlaid chromatograms are shown of the MRM EICs for a PGI6 standard solution, and a bupivacaine sample solution spiked with 1 ppm PGI6. The PGI elutes closely to the overloaded and tailing bupivacaine peak. The end time of the bupivacaine peak as detected by

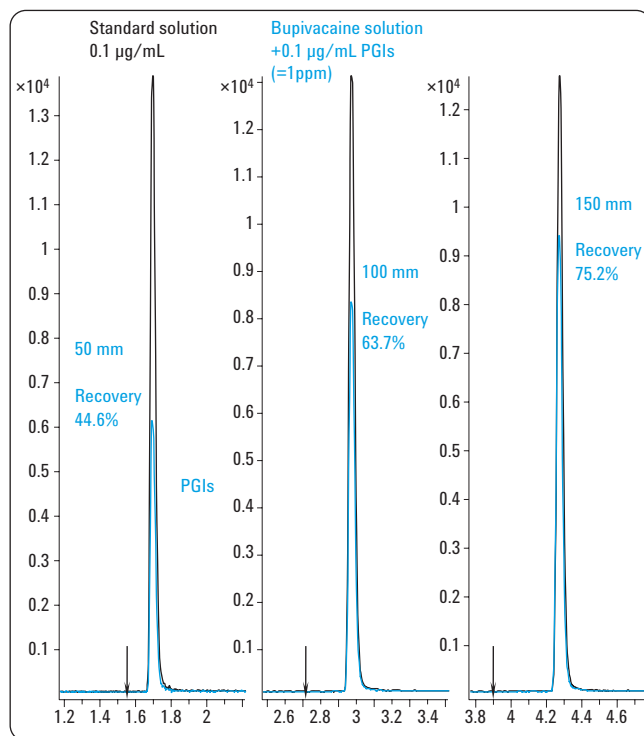


Figure 5
Comparison of the analysis of PGI6 in a standard solution and a spiked bupivacaine solution on different column lengths. Modifier: acetonitrile, detection: MS, 150.1>108.0 m/z. Arrow marks end time of the bupivacaine peak as detected by the DAD.

the DAD is marked with the arrow in Figure 5. Using a 50 mm column, PGI6 elutes too close to the API and the response is only 45% of the response of the same amount injected in a standard solution. On a 100 mm column, resolution between API and PGI6 increased, as well as recovery (accuracy) (to 64%). On the 150 mm column, resolution and accuracy further increased (recovery > 75%). This example clearly illustrates the role of high resolution HPLC for trace analysis, especially when a generic method is used.

Selectivity tuning

Next to column length, resolution (and consequently accuracy of PGI determination) can also be enhanced using selectivity tuning. The selectivity was varied by changing the organic modifier (acetonitrile or methanol). The influence of the organic modifier on selectivity can be very significant, depending on the application. The modifier is a method parameter and is therefore more easily changed during method development than the buffer composition or pH. The influence

of using methanol or acetonitrile on the separation is well illustrated when analyzing PGI10 in a diclofenac solution. PGI10 is a known impurity of diclofenac. The obtained DAD chromatograms (296 nm) are shown in Figure 6, which shows the comparison between analysis of a spiked and non-spiked diclofenac solution, using methanol or acetonitrile as organic modifier. When using methanol, an impurity originating from the drug substance coelutes with PGI10 at 6.25 min. This could lead to false positive results or to over-estimation of the amount of PGI10 when no precautions are taken. Replacing the methanol with acetonitrile leads to baseline separation between the two impurities and shows that no detectable amount of PGI10 is present in the diclofenac solution.

Method performance on different APIs

The four selected APIs were analyzed, before and after spiking with the 10 PGIs at 0.1 ppm level. The recovery in a sample solution for each impurity was compared to a standard mixture with the same PGI concentration (= accuracy = (response in sample/response in standard)*100). The analyses were carried out on 50, 100, and 150 mm columns with methanol and acetonitrile (six methods). PGI10 was detected with DAD at 238 and 296 nm. An overview of the results is shown in Table 3. The retention time for the PGIs and the retention time window for the APIs are also shown in the table. Grey areas indicate that the PGI is coeluting with the API under the given conditions and, consequently, the recoveries are very low. Since lidocaine and chlorhexidine contained significant quantities of the respective PGIs 9 and 8, no recovery could be calculated. The lidocaine product contained more than 2 ppm of PGI9 while PGI8 was found in chlorhexidine at a level above 20 ppm.

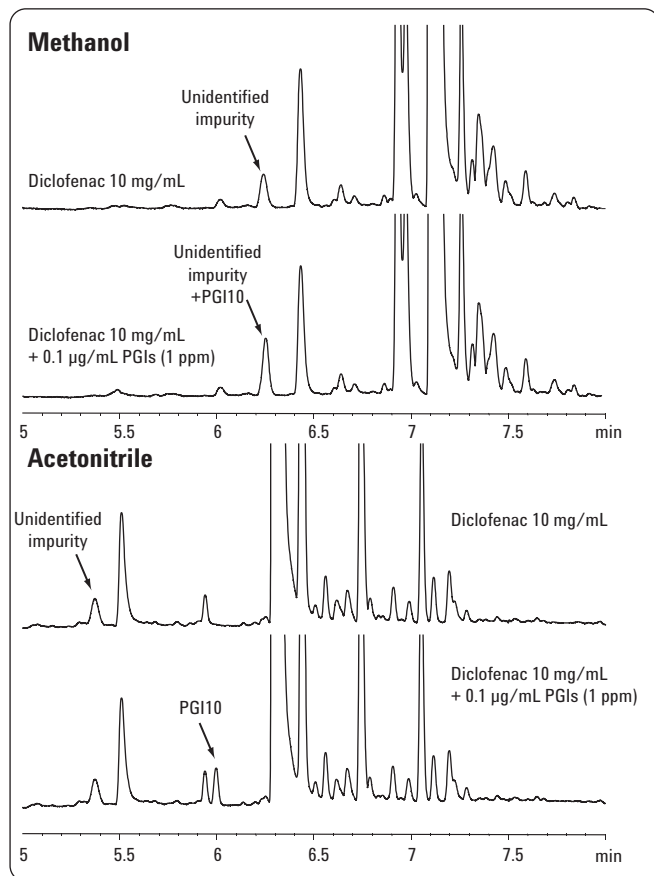


Figure 6
Comparison of the analysis of PGI10 in a spiked and non-spiked diclofenac solution with methanol (top) or acetonitrile (bottom) as modifier. Column length: 150 mm, detection: DAD 296 nm.

		PGI1	PGI2	PGI3	PGI4	PGI5	PGI6	PGI7	PGI8	PGI9	PGI10 238 nm	PGI10 296 nm	
0.1 ppm ACN, 50 mm		Retention time	1.50	1.80	1.30	1.15	1.90	1.70	1.25	1.50	1.60	2.25	2.25
Lidocaine	0.65-1.30	22.5	38.7	0.0	0.0	52.8	32.6	0.0	16.3	(a)			
Bupivacaine	1.35-1.55	0.0	58.4	78.5	81.3	73.1	43.5	78.8	0.0	0.0			
Chlorhexidine	1.45-1.55	4.4	81.5	86.7	70.0	51.8	64.3	78.0	(a)	80.1			
Diclofenac	>2.30	86.7	90.4	97.8	118.4	62.5	96.3	92.7	101.1	94.7	77.9	94.8	
ACN, 100 mm		2.60	3.20	2.25	1.95	3.40	3.00	2.15	2.60	2.80	4.10	4.10	
Lidocaine	1.40-2.25	43.1	72.6	0.0	0.0	69.4	51.3	1.3	35.6	(a)			
Bupivacaine	2.35-2.70		83.4	83.5	82.5	91.2	64.3	83.4	0.0	52.8			
Chlorhexidine	2.50-2.70	2.3	91.6	94.6	65.0	53.3	80.6	61.8	(a)	91.9			
Diclofenac	>4.25	88.3	96.1	94.7	95.0	61.4	94.2	93.3	104.2	97.6	88.8	87.7	
ACN, 150 mm		3.70	4.60	3.20	2.75	4.90	4.30	3.10	3.70	4.05	6.00	6.00	
Lidocaine	2.10-3.15	63.6	73.5	0.0	0.0	85.1	64.5	1.6	45.4	(a)			
Bupivacaine	3.50-3.90	0.0	84.6	80.6	105.5	108.6	81.0	89.5	0.0	66.5			
Chlorhexidine	3.65-3.95	60.0	92.8	101.6	77.9	58.3	89.4	73.0	(a)	94.3			
Diclofenac	>6.20	104.2	91.6	101.9	82.6	60.6	102.9	95.8	106.0	101.3	112.5	136.5	
MeOH, 50 mm		1.35	1.75	1.35	1.10	2.20	1.90	1.25	1.40	1.65	2.35	2.35	
Lidocaine	0.65-1.40	0.0	38.5	0.0	0.0	56.3	38.5	0.0	0.0	(a)			
Bupivacaine	1.50-1.85	74.0	0.0	88.2	99.6	72.1	33.9	82.2	97.0	0.0			
Chlorhexidine	1.75-1.95	82.4	78.7	93.9	74.5	44.5	0.0	82.3	(a)	86.1			
Diclofenac	>2.55	98.4	92.3	100.1	108.3	65.0	95.2	99.6	67.7	95.4	(b)	(b)	
MeOH, 100 mm		2.40	3.10	2.35	1.85	3.95	3.40	2.15	2.45	2.90	4.30	4.30	
Lidocaine	1.35-2.40	29.0	49.2	0.0	0.0	74.7	56.7	0.4	6.5	(a)			
Bupivacaine	2.75-3.20	99.9	0.0	94.4	102.8	91.9	62.8	95.0	96.2	0.0			
Chlorhexidine	3.20-3.45	86.2	98.9	107.3	103.0	52.2	6.4	75.9	(a)	107.5			
Diclofenac	>4.70	107.3	105.8	107.3	112.6	67.7	99.7	101.8	107.2	108.0	(b)	(b)	
MeOH, 150 mm		3.40	4.45	3.40	2.65	5.75	4.90	3.10	3.50	4.15	6.25	6.25	
Lidocaine	2.00-3.45	30.5	77.2	0.0	0.0	82.7	55.1	0.0	0.0	(a)			
Bupivacaine	4.05-4.65	85.7	0.0	77.1	111.0	94.8	56.3	90.6	87.4	8.6			
Chlorhexidine	4.55-5.00	85.3	126.1	84.4	128.7	84.5	3.2	75.7	(a)	97.2			
Diclofenac	>6.90	78.3	102.4	86.2	109.1	59.7	97.0	97.7	99.9	97.5	(b)	(b)	

(a) Significant quantity present in API. No recovery calculation.

(b) PGI10 coelutes with other impurity. No data available for DAD (see also Fig 6)

Table 3
Results (recovery, %) for API solutions spiked with 0.1 ppm PGIs. Grey areas indicate that the PGI is coeluting with the API under the given condition.

An example of the analysis of a chlorhexidine solution spiked with the PGIs at the 1 ppm level is shown in Figure 7. In the DAD chromatogram a large peak is detected for the API between 4.5 and 5 min together with a series of impurities and related substances. Also the extracted ion chromatograms from the MRM data acquisition are given. In these traces, PGIs 1 to 9 are detected. The recoveries calculated by comparison of a standard solution of the PGIs and the spiked sample solution are shown between brackets in the figure. For most PGIs good results are obtained. Accuracy is between 70% and 130%, which are typical limits in pharmaceutical trace analysis work (limit tests). PGI8 is present in the original API at a concentration significantly higher than the spiked concentration. Therefore it is not possible to determine the recovery for this solute in this specific sample. For PGI6, which partially coelutes with the API, however, a significantly lower recovery (accuracy) is obtained due to significant ion suppression.

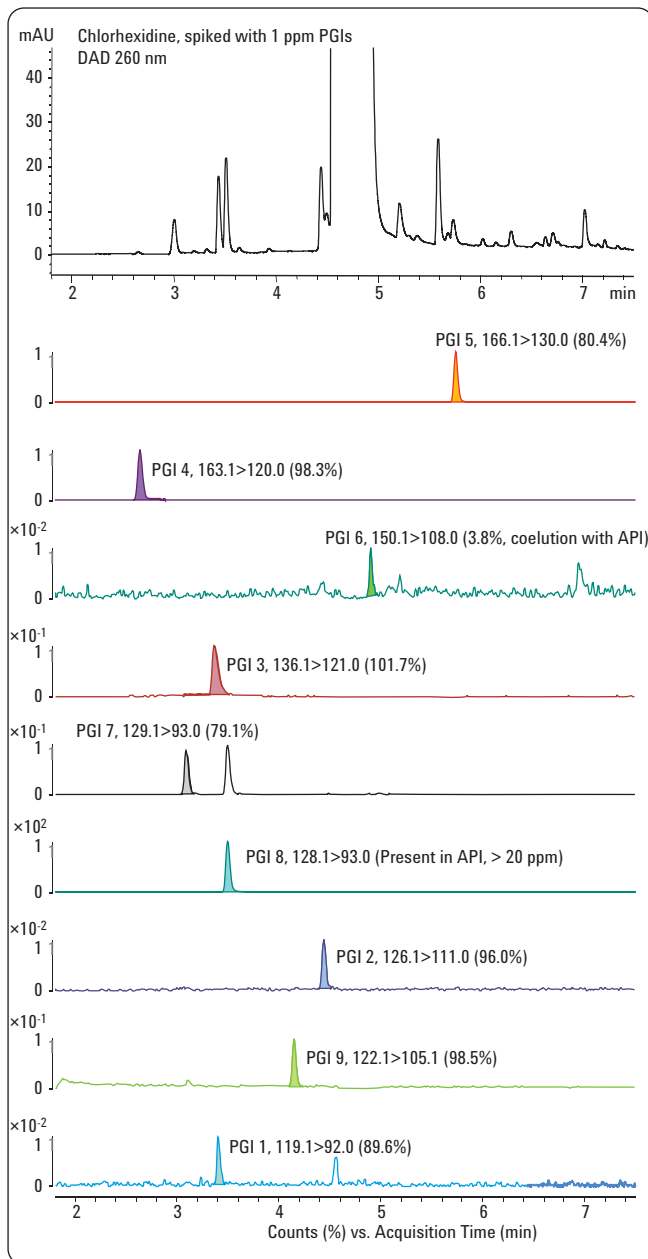


Figure 7
DAD result and Quantifier transitions of the analysis of a chlorhexidine sample spiked with 1 ppm PGIs. Column length: 150 mm, modifier: methanol. Transitions and calculated recoveries are indicated.

Selectivity tuning was needed. The analysis was repeated by replacing methanol by acetonitrile. The MRM transition chromatogram obtained using this method was compared to the chromatogram obtained using methanol in Figure 8. The same sample was injected and it is clearly observed that PGI6 was recovered using the new method. This example shows that the approach of selectivity tuning works very well.

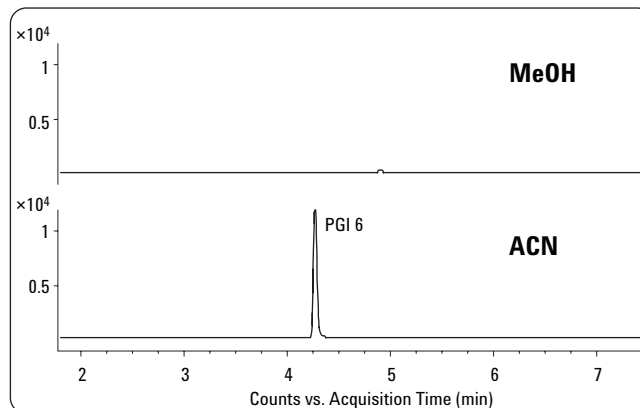


Figure 8
Determination of PGI6 in chlorhexidine (spiked at 0.1 ppm), 150 mm column, methanol (top) or acetonitrile (bottom) modifier.

Another example of selectivity tuning by changing the organic modifier is shown in Figure 9 for the analysis of PGI1 in bupivacaine. The MRM transitions of a standard solution and a sample solution, using acetonitrile (Fig 8A and B) and methanol (Fig 8C and D) illustrate that the presence of the API (eluting at 2.35-2.70 min on the 100 mm column using acetonitrile) completely suppresses ionization for PGI1. Using methanol, PGI1 is resolved from bupivacaine and detection at trace level is possible. In this case, methanol was better than acetonitrile.

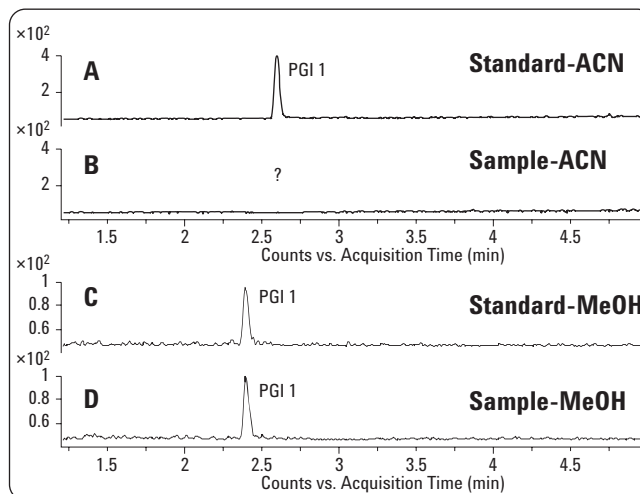


Figure 9
Determination of PGI1 in bupivacaine (spiked at 0.1 ppm), 100 mm column.

Increasing analysis speed

The analysis speed and sample throughput can be drastically increased using a short column, if sufficient resolution is obtained. This is first illustrated for the analysis of PGI10, performed by UHPLC using DAD detection. From the chromatograms shown in Figure 3, it is clear that acetonitrile produces the best resolution. Using a 50 mm column and faster (scaled) gradient, the analysis time can be reduced by a factor of 3. This is illustrated in Figure 10. PGI10 can be detected at 1 ppm level in the sample.

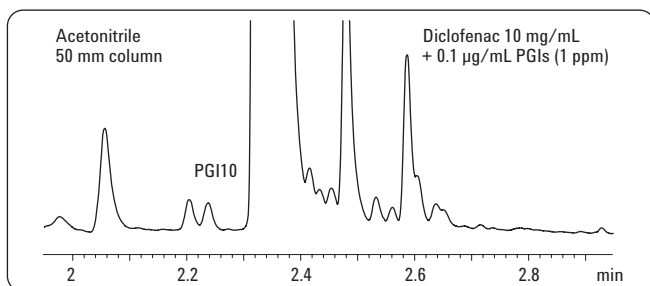


Figure 10
Determination of PGI10 in diclofenac (spiked at 1 ppm), 50 mm column, ACN modifier, DAD detection.

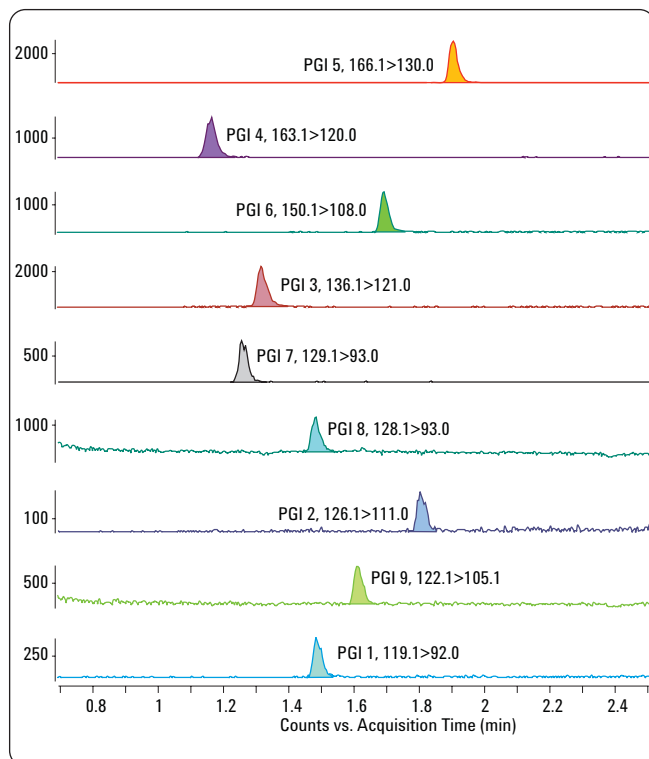


Figure 11
Determination of PGIs 1-9 in diclofenac (spiked at 0.1 ppm), 50 mm column, ACN modifier.

Using the same fast UHPLC method on a 50 mm column, the other PGIs can also be detected in the same sample using MRM MS/MS as illustrated in Figure 11.

Conclusion

The Agilent 1290 Infinity LC system and Agilent G6460A Triple Quadrupole LC/MS system can analyze for the selected arylamine and aminopyridine PGIs at levels well below 1 ppm relative to the API. Variations of organic modifier and column length can be used to tune the selectivity and peak capacity/resolution. This generic approach can be used in early method development stages or during screening procedures prior to method optimization. Detection limits are below 20 ppb (relative to the API) using MS/MS and below 100 ppb (relative to the API) with DAD for 2,6-dichloroaniline, which can not be detected with MS at these levels.

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