Identification and Quantitation of Co-eluting Non-steroidal Anti-inflammatory Drugs by Diode Array Detection

LC

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Introduction

HPLC separations are often performed in less than 5 minutes using columns that are only 3-cm in length. These short columns are ideal for rapid method development because they significantly decrease analysis time, equilibration time and solvent consumption. Column efficiencies of ~5,000 theoretical plates are obtained for a 3 cm x 4.6 mm i.d. column packed with 3-μm particles (compared to ~10,000 theoretical plates for a 15 cm x 4.6 mm i.d. column packed with 5-μm particles). Very short columns do give much faster separations compared to standard columns, but obviously some efficiency is sacrificed in the process. Therefore, complex mixtures may not be fully resolved by a short 3-cm column; some components may overlap or coelute. Using a longer column would increase the number of theoretical plates and solve the resolution problem, but the analysis time would be increased even more. An alternative approach is to run fast HPLC without resolving all the components chromatographically, and use a selective detection method to identify and quantitate each component present in the mixture.

In this application note, mixtures of 10 non-steroidal anti-inflammatory drugs (NSAIDs) were analyzed in less than 4 minutes by fast HPLC with diode array detection. Varian's PolyView multicomponent analysis (MCA) software was used to identify and quantitate all 10 drug components present in the mixtures, even when 3 of those drugs coeluted. MCA can identify and quantitate up to 6 coeluting compounds.

Experimental

A Varian LC Star System was used, consisting of:

- Varian 9012 ternary gradient pump
- Varian 9100 AutoSampler
- Varian Polychrom 9065 Diode Array Detector
- Varian Star LC Workstation software with PolyView version 2.02 diode array software
- Column: 3.3 cm x 4.6 cm Supelcosil LC-8DB, 3 μm particle size (Supelco, USA)
- Mobile Phase: Isocratic; 52% methanol/48% 50 mM KH2PO4; pH 6.0; 1 mL/min
- Sample: 10 μL injected of each NSAID standard mixture, A and B (**See Table 1**)

Results and Discussion

Each of the 10 drugs was diluted to a known concentration and then run individually by HPLC to obtain retention time data, response factors, spectral and purity parameter data (PuPs) for each drug. An NSAID spectral library consisting of spectral data for all 10 drugs was created by the PolyView software and saved for later automated or manual use.

Table 1 lists the NSAIDs used in this work, their individual HPLC retention times and peak apex PuPs. Ibuprofen and oxaprofen had almost identical retention times and would coelute if both were present in a mixture. However, the PuP values of ibuprofen and oxaprofen were 223.112 and 255.069, respectively, indicating that their spectra differed significantly.

Indomethacin eluted only 0.3 minutes later than the ibuprofen/oxaprofen peak, and had a PuP value of 255.873. The normalized library apex spectra of ibuprofen, oxaprofen, and indomethacin are shown in **Figure 1**.

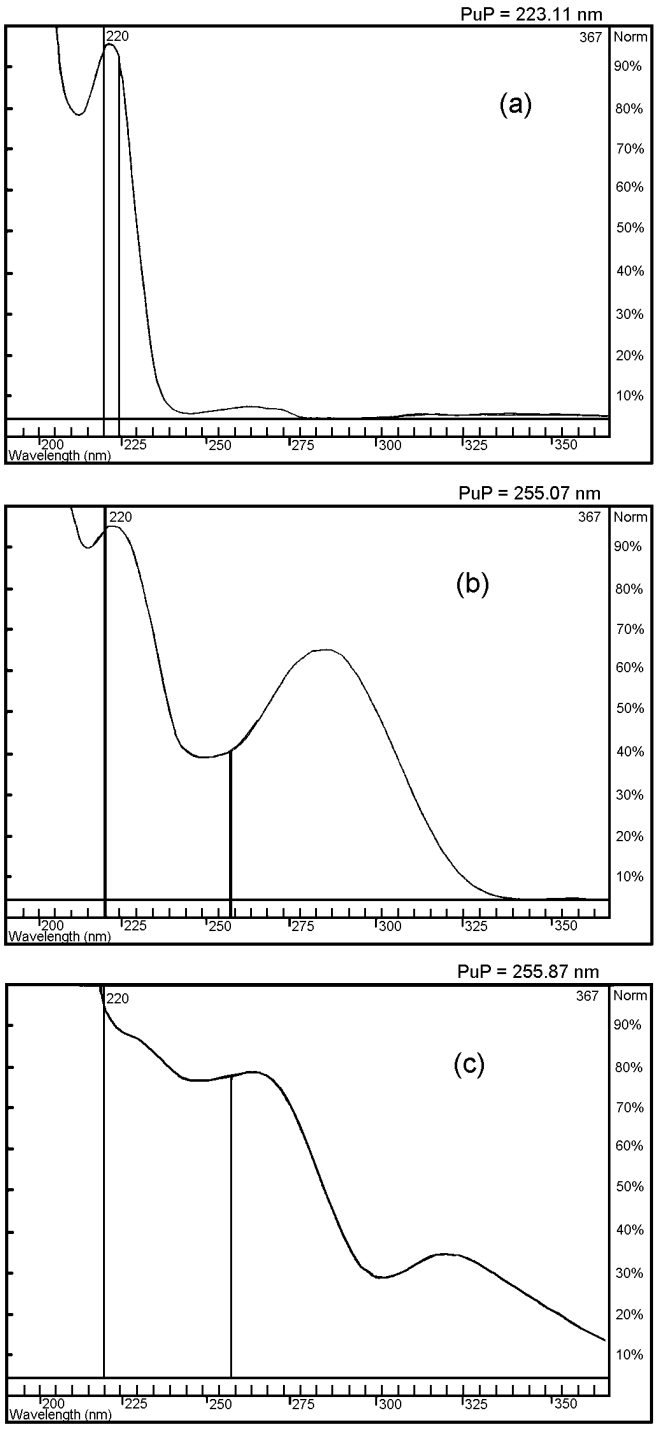

Figure 1. Library apex spectra of: (a) Ibuprofen, (b) oxaprofen, and (c) indomethacin. Obtained from individual HPLC chromatograms of each pure standard

NSAID mixtures containing these three co-eluting drugs (ibuprofen, oxaprofen, and indomethacin) would usually be difficult to quantitate at a single wavelength under these HPLC conditions. A separation could be achieved if a longer more efficient column were used, but then the advantage of speed would be lost.

Analysis of Standard Mixture A

A standard mixture A was made up from 9 NSAID drugs at the concentrations listed in **Table 2.** This standard contained 5.00 ng/µL of ibuprofen and 0.50 ng/µL of oxaprofen, but did not contain indomethacin. The HPLC chromatogram of Standard A at 220 nm is shown in **Figure 2**. Note that ibuprofen (peak 7) and oxaprofen (peak 8) coelute, while all the other components present in the standard are almost baseline resolved. The quantitative results of this separation using a single wavelength (220 nm) are shown in the fourth column of Table 2. Only ibuprofen was detected at 5.82 ng/µL (it should have been only 5.00 ng/µL); oxaprofen was not detected as a separate peak but obviously contributed to the higher than expected result for ibuprofen.

Multicomponent analysis (MCA) diode array software was then used to quantitate the coeluting NSAIDs in Standard A, ibuprofen and oxaprofen, based on the differences in the individual spectra of these drugs. **Figure 3** shows the multicomponent spectrum analysis of fused peak 7+8 over the 220-325 nm wavelength range. The total spectrum of the fused peak is shown as a combination of the individual spectra of ibuprofen and oxaprofen.

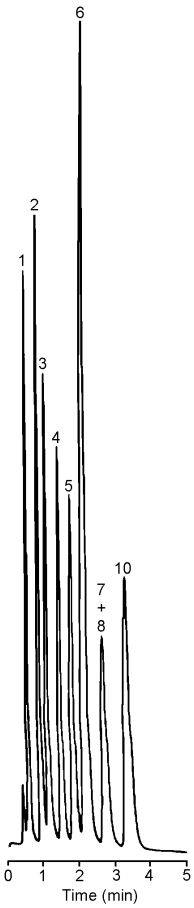

Figure 2. HPLC chromatogram of standard mixture A at 220 nm. Peak numbers as in Table 1

Table 1. HPLC retention times and UV spectral purity parameters (PuP) of the 10 NSAIDs used in this study

Peak No.	NSAIDs	HPLC Retention Time (min)	Peak Apex PuP (220-367 nm)
1	Piroxicam	0.58	299.968
2	Naproxen	0.91	228.651
3	Fenpipalone	1.11	242.691
4	Diftalone	1.50	243.319
5	Cicloprofen (IS)	1.86	262.512
6	Carprofen	2.22	244.701
7	Ibuprofen	2.77	223.112
8	Oxaprofen	2.78	255.069
9	Indomethacin	3.01	255.873
10	Fenclofenac	3.47	227.986

Table 2. Theoretical and calculated concentrations of NSAID standard mixture A

Peak No.	NSAIDs	Theoretical Conc. (ng/µL) of Std A	Calc. Conc. (ng/µL) of all peaks in Std A (no MCA)	Calc. Conc. (ng/µL) of fused peaks in Std A (using MCA)
1	Piroxicam	10.00	10.02	10.02
2	Naproxen	2.00	2.02	2.02
3	Fenpipalone	10.00	10.01	10.01
4	Diftalone	7.50	7.48	7.48
5	Cicloprofen (IS)	5.00	INT STD	INT STD
6	Carprofen	7.50	7.47	7.47
7	Ibuprofen	5.00	5.82	4.99
8	Oxaprofen	0.50	not detected	0.47
9	Indomethacin	0.00	not detected	not detected
10	Fenclofenac	5.00	5.06	5.06

Figure 4 is the multicomponent plot analysis (MCA) of fused peak 7+8 shown at 220 nm, showing the relative contributions of ibuprofen and oxaprofen to the total peak. Quantitation by this MCA method yielded the results shown in the last column of Table 2, i.e., 4.99 ng/µL ibuprofen and 0.47 ng/µL oxaprofen. These results compared very well with the theoretical values expected for Standard A.

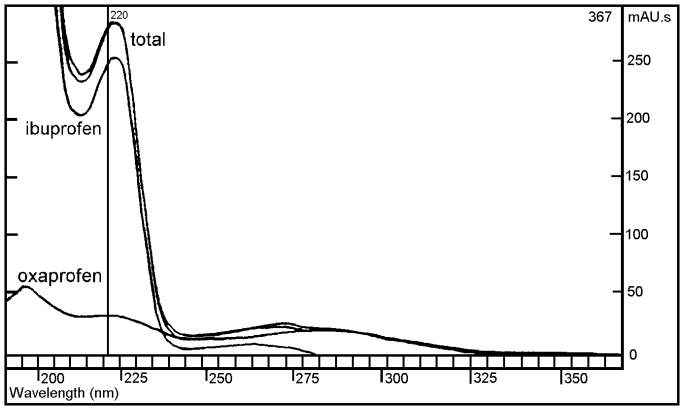

Figure 3. Multicomponent spectrum analysis of fused peaks 7 and 8 in standard mixture A, over 220-325 nm

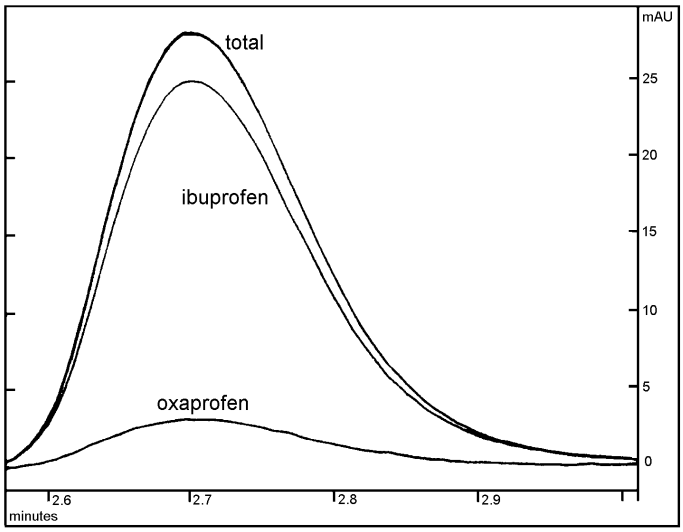

Figure 4. Multicomponent plot analysis of fused peaks 7 and 8 in standard mixture A at 220 nm

Analysis of Standard Mixture B

Standard B was made from 10 NSAIDs including 5.00 ng/μL of ibuprofen, 1.00 ng/μL of oxaprofen, and 5.00 ng/μL of indomethacin (**Table 3**). The HPLC chromatogram of Standard B detected at 220 nm is shown in **Figure 5**.

Indomethacin (peak 9) is only partially separated from the coeluting ibuprofen/oxaprofen peak (7+8), enough to detect it but not enough to provide accurate quantitation for all three components, 7-9. Table 3 shows the concentrations calculated from single wavelength data in column 4:

Table 3. Theoretical and calculated concentrations of NSAID standard mixture B

Peak No.	NSAIDs	Theoretical Conc. (ng/μL) of Std B	Calc. Conc. (ng/μL) of all peaks in Std B (no MCA)	Calc. Conc. (ng/μL) of fused peaks in Std B (using MCA)
1	Piroxicam	10.00	10.15	10.15
2	Naproxen	3.00	3.01	3.01
3	Fenpipalone	10.00	10.11	10.11
4	Diftalone	10.00	9.77	9.77
5	Cicloprofen (IS)	5.00	INT STD	INT STD
6	Carprofen	5.00	5.00	5.00
7	Ibuprofen	5.00	5.75	4.94
8	Oxaprofen	1.00	not detected	1.05
9	Indomethacin	5.00	5.35	5.03
10	Fenclofenac	3.00	3.05	3.05

Oxaprofen was not detected; ibuprofen was detected at 5.75 ng/μL; and indomethacin was detected at 5.35 ng/μL. However, this chromatographic data was good enough to quantitate the other 7 resolved NSAID components, using cicloprofen as the internal standard. When MCA was applied to the separation of Standard b using the same data but over 220-362 nm wavelength range, oxaprofen was detected. **Figure 6** shows how the spectra of the individual components 7-9 present in the fused peak contribute to the total spectrum obtained. The spectrum for oxaprofen can be clearly seen near the bottom. MCA yields a plot **(Figure 7)** made from the relative contributions of ibuprofen, oxaprofen and indomethacin, and the numerical results of this are given in the last column of Table 3: 4.94 ng/μL ibuprofen, 1.05 ng/μL oxaprofen, and 5.03 ng/μL indomethacin. This compares very well to the injected amounts.

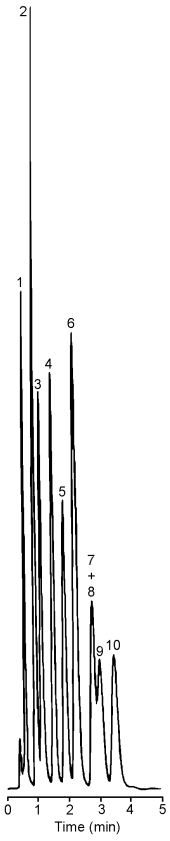

Figure 5. HPLC chromatogram of standard mixture B at 220 nm. Peak numbers as in Table 1

Conclusions

Varian's PolyView MCA software is highly effective at identifying and accurately quantitating coeluting drugs from their spectral data. When time constraints are imposed on either the development of a method or on the analysis itself, complete resolution of peaks may be an unnecessary luxury. In such cases, MCA can be used to analyze fast HPLC data without losing the ability to quantitate accurately.

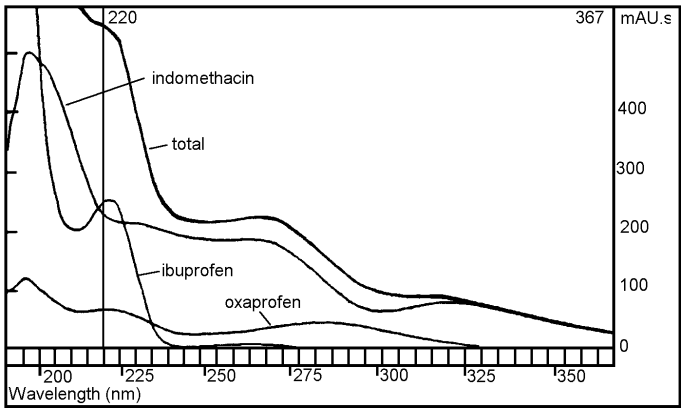

Figure 6. Multicomponent spectrum analysis of fused peaks 7, 8 and 9 in standard mixture B, over 220-362 nm

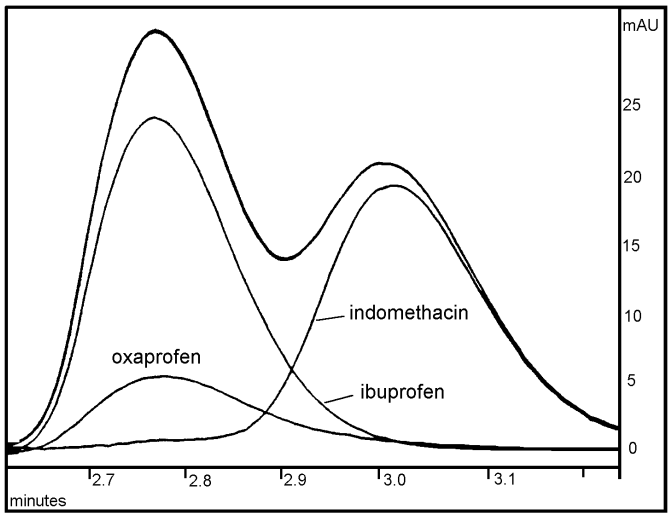

Figure 7. Multicomponent plot analysis of fused peaks 7, 8 and 9 in standard mixture B at 220 nm