

Standard C₁₈ column in high throughput LC-MS applications

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Introduction

In today's paradigm of drug discovery, high throughput screening of hit-to-lead compounds is commonplace. Researchers screen millions of chemical compounds found in commercially available and proprietary libraries in search of a lead molecule, which reacts in a pre-defined biochemical process, and thus possesses the potential to become a FDA approved drug. Production of these huge chemical libraries begins in the arena of synthetic chemistry and exploits the techniques of combinatorial chemistry. The creation of these libraries solely for the intention of drug discovery places a large burden on the analytical chemist. Each library must be screened to provide the medicinal researcher insight into the identity and purity of each component. The job of the analytical chemist is therefore to design a method that will enhance the likelihood of accurate results and facilitate the throughput needed to support the synthetic processes.

As described previouslyⁱ the variables that affect the length of the chromatographic run-time are (in order of increasing effect): column temperature, organic gradient steepness, instrument delay (dwell) volume, flow rate, and column length. Of these variables, the prudent choice of column composition, particle size, diameter and length is paramount. Shorter columns with smaller particles dramatically decrease analytical run time and can often maintain or increase resolution when compared to longer columns with larger particles. With these factors in mind the following method was developed:

COLUMN: SMT OD-5-100/3 C₁₈; oven (30°C)
HPLC: Gradient = 98% A to 96% B (Linear) over 0.45 min
A: 25mM NH₄OAc in H₂O
B: 50/50 CH₃CN/MeOH
DAD (210nm – 300nm)
MS:
ES +/- switching
Cone: 25V
Mass range: 160-700da

Experimental

Today, many models exist to predict the bioavailability of a potential drug candidate. Lipinski's Rule of Fiveⁱⁱ has been extensively used for this purpose, and the parameters (see Table 1) of the Lipinski "Rule" have been demonstrated as common characteristics in most commercial pharmaceuticals available today. The Rule of Five derives its name from the fact that the relevant cutoffs are multiples of five and expound that a potential drug candidate will possess the following physicochemical characteristics: 1) the sum of OH and NH groups (hydrogen bond donors) on the molecule should be less than or equal to five 2) the sum of the N and O atoms (hydrogen bond acceptors) on the molecule should be equal to or less than ten 3) the molecular weight should not exceed 500da and 4) the octanol / water partition coefficient (LogP) should be less than five.

Approximately 10% of a commercially available High-Throughput Screening (HTS) library (ASDI Biosciences, Inc. Newark, DE) was chosen for this study and resulted in a sample set of 10,000 compounds. Table 1 illustrates the physicochemical properties of the sample set in light of Lipinski's Rules.

Table 1. Physico-chemical properties of the HTS library sample subset.

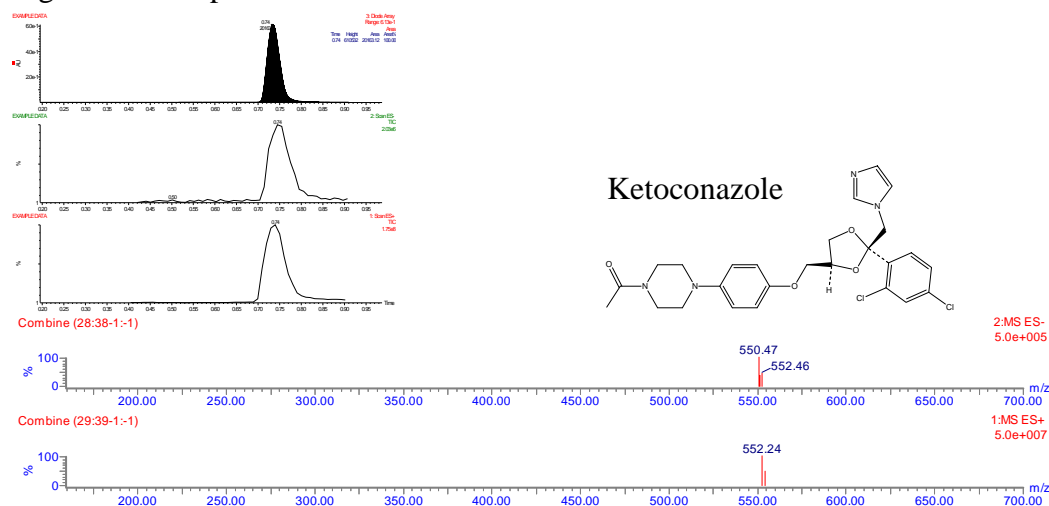
Physicochemical properties		Rule of 5 Violations	
MW > 500	17.60%	0	6047
Average MW	435.8	1	2644
CLogP > 5	35.10%	2	1309
Average CLogP	4.35		
Hbond Acceptors > 10	0%		
Average Hbond Acceptors	5		
Hbond Doners > 5	0%		
Average Hbond Donors	1		

Two identical LC-MS systems were set up according to the method described above, and the 10,000 compound subset of the HTS library was analyzed over a consecutive 6-day period. The method demonstrated the ability to analyze more than 850 samples per 24-hour period per instrument.

Results

Of the 10, 000 compounds that comprised the sample set, 100% were positively identified by electrospray positive and/or negative mass spectrometry with an average relative purity of 98%. Figure 1 illustrates an example of the data. Column lifetimes averaged approximately 3500 injections or more, and no in-line filter or guard column was used before the columns in all cases.

Figure 1. Example data.



Conclusion

The ability to rapidly and precisely assess the quality of large and diverse chemical libraries is absolutely essential in today's high-throughput milieu. Analytical methods must be developed to meet the demand, and the LC-MS method described herein demonstrates the potential to "rise to the occasion". By choosing the SMT OD-5-100/3 C₁₈ column, one overcomes the most critical obstacles that limit analytical run time and resolution and facilitates rapid semi-quantitative analysis of the drug-like compounds that comprise hit-to-lead libraries at a rate of greater than 800 compounds per day per instrument. This type of an approach and application is readily applicable to other situations where high throughput LC-MS analysis is needed.

ⁱ Waeghe, T. Will a shorter HPLC column work for my application? Separation Times 2003 16:3, 19-21

ⁱⁱ CA Lipinski, *Adv. Drug Del. Rev.* 1997, 23, 3