FITTING KINETIC EQUATIONS DERIVED FROM METABOLIC PATHWAYS TO LC/MS-MS INTEGRATED PEAK AREAS

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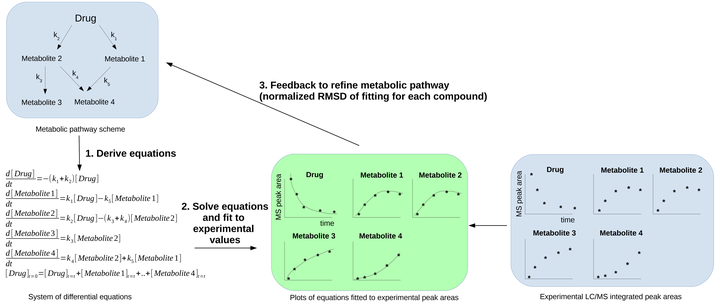
Overview

We describe a method to verify if LC/MS data from *in vitro* incubations with microsomes agree with a proposed metabolic pathway, and also to guide the expert when building a metabolic pathway. In this method, implemented in WebMetabase (Molecular Discovery, Ltd. UK), we build and solve a system of chemical kinetics differential equations from chemical kinetics derived from a metabolic pathway of a drug, and fit the solved time-dependent equations to the MS peak areas of drugs incubated with liver microsomes and analyzed through LC-MS.



Methods

This methodology has several requirements and assumptions:

- Samples must be obtained at different incubation times
- Incubations are single-compartiment (typical for *in vitro* incubations with microsomes)
- Arrows in a metabolic pathway are treated like single-step, first order, irreversible chemical reactions
- The amount of metabolites is zero at incubation time = 0

Using chemical kinetics theory, we build a system of ordinary differential equations (ODEs) describing the rate of change of the concentration of each compound in the metabolic pathway. The system of ODEs is solved, obtaining for each compound in the metabolic pathway one equation that relates its concentration with time. Usually there is a linear relationship between concentration and MS peak area, therefore we can apply them to describe MS peak areas as well, and find the parameters that best fit the equation for each compound to the values of its integrated MS peak areas. We provide a **normalized root mean squared deviation (RMSD)** value as an assessment of goodness of fit for each compound:

$$\text{Normalized RMSD} = \frac{\sqrt{\sum_{i=1}^{N}\left(y_i^{exp} - y_i^{theor}\right)^2 / N}}{Y_{max} - Y_{min}}$$

y_i^{exp} = Experimental peak area
y_i^{theor} = Fitted equation peak area
N = Number of samples
Y_{max}-Y_{min} = Maximum difference in observed peak areas

RMSD values are normalized for each compound, thus a normalized RMSD of 0.05 can also be expressed as an error in the fitting of 5% of the maximum range of observed peak areas for that compound.

Results

We applied this method on human liver microsomes incubations with dextromethorphan (Fig. 1) and buspirone (Fig. 2), for which metabolic pathways have been published.

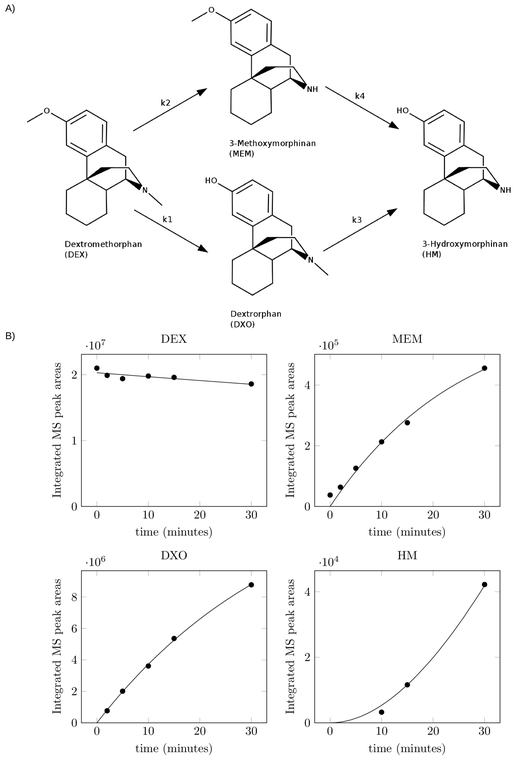


Figure 1. A) Metabolic pathway showing dextromethorphan and its major metabolites. B) Experimental MS peak areas (dots) and fitted solved equation (line) for each compound shown in dextromethorphan metabolic pathway.

All equations for compounds in the metabolic pathway of dextromethorphan (Fig. 1A) showed good fitting with experimental peak areas, with normalized RMSD values lower than 0.04 in all cases.

The metabolic pathway of buspirone (Fig. 2A) also showed good fitting for many compounds (Fig. 2B), with the worst fittings observed for M1, M23, M7 and M17 (with normalized RMSD= 0.0818, 0.118, 0.0995 and 0.101 respectively).

Fitted equations of all compounds with the same generation (i.e. distance to the parent drug) show similar shapes (compare plots in different colored boxes of Fig. 2B). Plots of fitted equations sometimes give a hint on whether the compound is further metabolized showing a low increase or even a decrease of peak areas at late incubation times (see M14 or M21 in Fig. 2B). Using this rationale we propose that M1, M23, M7 and M17 should be further metabolized into other compounds, that were not detected in our experimental conditions (dashed lines in Fig.2B show how would an equation fit the experimental values in these cases).

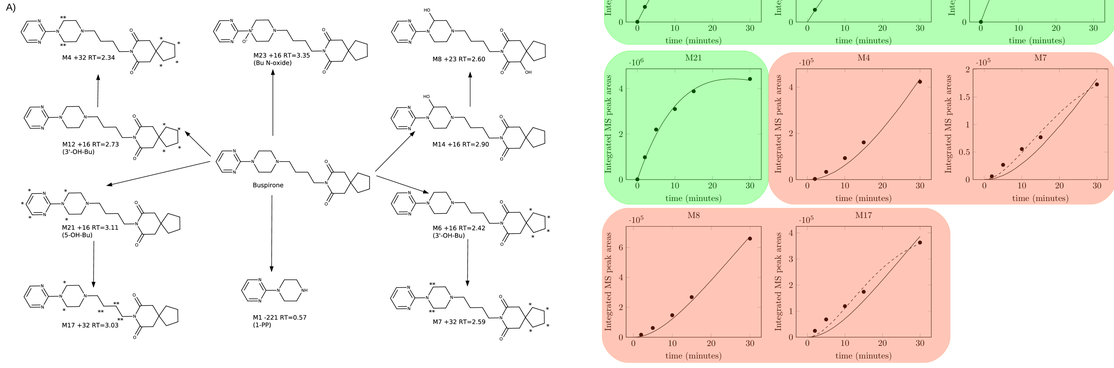


Figure 2. A) Metabolic pathway showing buspirone and its major metabolites (unresolved hydroxylation sites shown with one asterisk, when more than one such site exists hydroxylation is marked with two asterisks in the second site). B) Experimental MS peak areas (dots), fitted solved equation (line), fitted solved equation if compound were further metabolized (dashed line) into other metabolites. Fitting plots are grouped inside colored boxes indicating generation and/or further metabolism of compound (first generation metabolites without child metabolites in blue boxes, first generation metabolites which are further metabolized in green boxes and second generation terminal metabolites in orange boxes).

Conclusions

Fitting solved chemical kinetics equations (which relate concentration with time) on MS peak areas (which usually show a linear relationship with concentration) allowed us to assess if a proposed metbolic pathway was in agreement with experimental MS data. Visual inspection of the fitting plots also hints at the generation of the compound and sometimes also whether the compound is further metabolized. This approach has been recently implemented in WebMetabase (version 3.2.0, Molecular Discovery, Ltd., UK), an application to archive, process, report and explore metabolite identification (Met ID) data from different experimental sources [1].

[1] Cece-Esencan E.N.; Fontaine, F.; Plasencia, G.; Teppner, M.; Brink, A.; Pähler, A. and Zamora, I. **"Software-aided cytochrome P450 reaction phenotyping and kinetic analysis in early drug discovery."** Rapid Commun Mass Spectrom. (2016) 30(2):301-10.