ANTHRACYCLINE SPECTRA UNDER ANALYSES

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Introduction

The identification of 4 anthracycline molecules: Epirubicin (EPI), Daunorubicin (DAUNO), Doxorubicin (DOXO) and Idarubicin (IDA), is non-specific by analysis with the QCRx® spectrophotometer (in UV - Raman), requiring a systematic double check of the visual identification of the vial. In order to improve this control, an analysis of the spectra of these molecules is carried out using high-pressure liquid chromatography (HPLC) as a function of temperature and derivatisation, with the aim of checking whether they show spectral differences.

Objective

Analysis of anthracycline spectral data as a function of temperature and derivation of spectra

Materials & Methods

Analysis of anthracyclines
- High-Performance Liquid Chromatography (HPLC) method
- Shimadzu Nexera X2®, UV-Vis detector (190-700nm)
- Direct injection
- Labsolutions® software

Development of a specific library
- Spectral library consisting of 8 samples per anthracycline of increasing concentration
- At 3 different temperatures (Ambient, 15°C and 10°C),
- At 3 derivatives (no derivative-SD, first derivative-1D and second derivative-2D).
- Sample diluted with 0.9% NaCL
- Products used :
  - Idarubicin 1mg/ml – PFIZER®
  - Epirubicin 2mg/ml – MEDAC®
  - Daunorubicin 5mg/ml – SANOFI®
  - Doxorubicin 2mg/ml – ACCORD®

Spectrum analysis
- Analysis of spectra acquired between 0.260 and 0.265min by similarity (lambda min/max) with variations in the method (temperature) and variations in analysis.
  - 6 identifications per sample analysed
  - 6 similarity scores

Statistical analysis
- A different sample identification is used to calculate the error rate (ER) per molecule.
- Statistical analyses are carried out using Excel® and XLStat®.

Results

The results are presented with : **The parameters tested** - **The results of the identification of the sample tested (6 results per sample) with the spectral library** - **The results of the similarity score (%RESS) of the sample with the spectra in the spectral library.**

Index	Temperature	Dérivé	DCI / Concentration / Fournisseur	ID 1	ID 2	ID 3	ID 4	ID5	ID6	RESS1	RESS2	RESS3	RESS4	RESS5	RESS6	V/F
214003	10°C	1ere	DAUNO	Daunorubi	Epirubicine	Epirubicine	Daunorubi	Daunorubi	Epirubicin	0,981507	0,918226	0,916445	0,907475	0,878794	0,86202	VRAI
214003	15°C	1ere	DAUNO	Daunorubi	Epirubicine	Daunorubi	Epirubicin	Daunorubi	Epirubicin	0,997396	0,992852	0,992091	0,98848	0,968584	0,963613	VRAI
214003	Ambiant	1ere	DAUNO	Daunorubi	Epirubicine	Doxorubici	Epirubicin	Daunorubi	Doxorubi	0,991529	0,99042	0,989121	0,983833	0,967408	0,961839	VRAI
214003	10°C	2nd	DAUNO	Daunorubi	Epirubicine	Daunorubi	Epirubicin	Daunorubi	Daunorubi	0,90643	0,724143	0,680598	0,580881	0,578759	0,468476	VRAI
214003	15°C	2nd	DAUNO	Daunorubi	Epirubicine	Daunorubi	Epirubicin	Daunorubi	Epirubicin	0,990112	0,975115	0,954424	0,949975	0,936335	0,93614	VRAI
214003	Ambiant	2nd	DAUNO	Daunorubi	Doxorubici	Epirubicine	Epirubicin	Epirubicin	Doxorubi	0,976873	0,905327	0,891876	0,887261	0,879315	0,878928	VRAI
214003	Ambiant	Sans dérivé	DAUNO	Daunorubi	Epirubicine	Daunorubi	Epirubicin	Daunorubi	Daunorubi	0,998891	0,998339	0,997917	0,997736	0,997654	0,997255	VRAI
214003	15°C	Sans dérivé	DAUNO	Daunorubi	Epirubicine	Doxorubici	Daunorubi	Daunorubi	Epirubicin	0,999518	0,998788	0,9983	0,998075	0,997852	0,997779	VRAI
214003	10°C	Sans dérivé	DAUNO	Daunorubi	Epirubicine	Doxorubici	Daunorubi	Epirubicin	Daunorubi	0,999078	0,998918	0,997384	0,997192	0,996849	0,996677	VRAI

→ 47 samples (DOXO=14, EPI = 14, DAUNO=8, IDA=7) were analysed for a total of 383 spectral data.

The ER by DCI, all temperatures and derivatives combined, obtained are :

	DOXO	EPI	DAUNO	IDA
TRUE	107	73	46	60
FALSE	30	49	17	3
TOTAL	137	122	63	63
ER (%)	22%	40%	27%	5%

Only **IDA shows ER at 0%**, particularly at room temperature and +15°C, whatever the derivative

➡ Additional analysis for IDA: A mean comparison test of the similarity scores (score of 1st compliant RESS=IDA vs score of 2nd non-compliant RESS=non IDA) revealed a significant difference between the 1st% and the 2nd%.

Difference	0,369
t (Observed value)	13,102
|t| (Critical value)	1,980
DDL	118
p-value (bilateral)	<0,0001
alpha	0,05

An ANOVA on repeated measures of the similarity score data 1st%RESS compliant=IDA vs 2nd%RESS non-compliant= non IDA revealed a more marked difference for the 1D and 2D spectra between the two scores: %

Derivative*Reco / Tukey (HSD) / Analysis of differences between modalities with a 95% confidence interval :				
Contraste	Difference	Standardised difference	Valeur Critq	Pr > Diff Significatif
Derivative-2nd*Reco-1R vs Derivative-2nd*Reco-2R	0,568	312,022	2,936	< 0,0001 Oui
Derivative-1rst*Reco-1R vs Déerivative-1rst*Reco-2R	0,460	253,076	2,936	< 0,0001 Oui
Derivative-No derivative*Reco-1R vs Derivative-No derivative*Reco-2R	0,057	31,212	2,936	< 0,0001 Oui

The ER, as a function of temperature and the derivative of the spectra, obtained are :

Derived	SD	1D	2D
TRUE	88	100	100
FALSE	39	28	28
TOTAL	127	128	128
ER (%)	31%	22%	22%

Chi² test negative. No significant difference between the different proportions = **no impact of the derivative on ER**

Temperature	Amb SD	15° SD	10° SD
TRUE	33	30	25
FALSE	13	11	15
TOTAL	46	41	40
ER (%)	28%	27%	38%

Negative Chi² test (p-value = 0,526)

Temperature	Amb 1D	15° 1D	10° 1D
VRAI	35	32	33
FAUX	11	9	8
TOTAL	46	41	41
ER (%)	24%	22%	20%

Negative Chi² test (p-value = 0,884)

Temperature	Amb 2D	15° 2D	10° 2D
TRUE	33	34	33
FALSE	13	7	8
TOTAL	46	41	41
ER	28%	17%	20%

Negative Chi² test (p-value = 0,410)

No significant difference between the different proportions = no impact of temperature on ER

Discussion & Conclusion

Analysis of HPLC spectral data for anthracyclines showed a TE and a statistically significant analysis only for IDA, which would eliminate the need for visual inspection of the vial. It is difficult to extrapolate the results to the control method used routinely, and further analysis is required.