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1 **Simultaneous quantification of dabrafenib, hydroxy-**
2 **dabrafenib and trametinib in human plasma by**
3 **liquid chromatography-tandem mass spectrometry**

4
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18 **Abstract**

19 A new liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the
20 simultaneous quantification of dabrafenib (DAB), its main metabolite hydroxy-dabrafenib
21 (OHD) and trametinib (TRA) in human plasma has been developed and validated. After
22 addition of internal standard (dabrafenib-d9), extraction was achieved after protein
23 precipitation with acetonitrile containing 1% (v/v) formic acid. Chromatographic separation
24 was performed on an Accucore[®] C18 (2.1 × 50 mm; 2.6 μm) column using a gradient elution
25 of water acidified with 0.1% (v/v) formic acid (A) and acetonitrile containing 0.1% (v/v)
26 formic acid (B) at a flow rate of 500 μL/min. The calibration ranged from 10 to 2000 ng/mL
27 for DAB and OHD and from 5 to 50 ng/mL for TRA. This method was validated with
28 satisfactory results including good precision (intra- and inter-assay coefficient of variation
29 from 2.0% to 14.9%) and good accuracy (inter- and intra-day bias between -1.2 % and 10.9
30 %), as well as long term stability in unprocessed plasma at -20°C. This newly proposed
31 method is useful for clinical research purposes as well as therapeutic drug monitoring for
32 patients with a Rapidly Accelerated Fibrosarcoma kinase B (BRAF)-mutated cancer.

33

34 Keywords: Therapeutic drug monitoring, Dabrafenib, Hydroxy-dabrafenib, Trametinib, Melanoma, LC-MS/MS

35 **1. Introduction**

36 Increased understanding of molecular pathways leads to the development of effective
37 targeted therapies for the treatment of solid tumors. Thus, a number of frequent driver
38 mutations (ie, genetic errors that control the disease) including Rapidly Accelerated
39 Fibrosarcoma kinase B (BRAF) have been identified in cancers such as melanoma and to a
40 lesser extent non-small cell lung cancer (NSCLC) and anaplastic thyroid cancer (ATC).
41 Targeted therapies in the form of signal transduction inhibitors, notably BRAF and MEK
42 inhibitors, have proven their efficacy in clinical trials [1]. Dabrafenib (DAB) and trametinib
43 (TRA) target the RAS-RAF-MEK-ERK transduction signal that regulates cellular growth and
44 survival. DAB is a selective and potent inhibitor of serine/threonine kinase BRAF^{V600E/K}
45 mutants, whereas TRA is a MEK inhibitor [2]. These two drugs have been approved by the
46 US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), alone
47 or in combination, for unresectable or metastatic melanoma, advanced NSCLC and ATC
48 [3,4].

49 DAB and TRA exhibit moderate to large interindividual variability in their
50 pharmacokinetics. Pharmacokinetic (PK) data from clinical trials highlight that predictive
51 factors such as age, sex or concomitant treatment can explain only in part this variability
52 which remains largely unexplained [2,5,6]. The magnitude of this PK variability could be
53 greater in unselected patients from “real life” who are more fragile than those included in
54 clinical trials for different reasons such as comorbidities, polymedication and age. Several
55 clinical studies reported higher plasma concentrations in patients experiencing dose-limiting
56 toxicities or specific toxicities such as pyrexia and fatigue for DAB [7]. The relationship
57 between plasma drug exposure and efficacy for DAB and TRA is currently controversial
58 [8,9], and requires further investigation. Moreover, DAB undergoes oxidative metabolism via
59 cytochrome P450 (CYP) 3A4 and CYP2C8 to form hydroxy-dabrafenib (OHD), an active

60 metabolite, with a twofold higher potency than an inhibitor of mutant BRAF. The mean
61 OHD-to-DAB exposure ratio in plasma is 0.8 [2]; therefore, the pharmacodynamic
62 contribution of OHD in clinical outcomes deserves to be investigated.

63 Several DAB and TRA liquid chromatography–tandem mass spectrometry (LC-
64 MS/MS) multi-analyte methods in human plasma have been described [10-16]. One of these
65 methods allows assaying simultaneously DAB and six metabolites (including OHD) in
66 plasma [17]. Because metabolite standards were not available, it is only semi-quantitative,
67 thus limiting its use to investigate pharmacokinetic/pharmacodynamic (PK/PD) relationship
68 for OHD. As far as we know, no quantitative method for OHD assay in plasma has been
69 reported yet.

70 In our laboratory, we recently conducted a PK/PD study in BRAF-mutated cancer
71 patients treated concomitantly with DAB and TRA [6]. For this purpose, OHD was custom
72 synthesized; then a LC-MS/MS method for OHD assay in plasma was developed to
73 investigate exposure–response relationship for toxicity and efficacy in BRAF-mutated
74 patients. The present study presents the development and the validation of a LC-MS/MS
75 method for the simultaneous quantification of DAB, OHD and TRA in plasma from BRAF-
76 mutated cancer patients.

77 **2. Materials & methods**

78 **2.1. Standards and reagents**

79 DAB (519.6 g/mol, purity $\geq 98\%$) and TRA (615.4 g/mol, purity $\geq 98\%$) were
80 purchased from Toronto Research Chemical (Toronto, Canada). AlsaChim (Illkirch, France)
81 provided the isotopically labelled internal standard dabrafenib-d9 (528.6 g/mol, purity $>98\%$).
82 OHD (535.6 g/mol, purity $>99\%$) was synthesized by Shanghai Medicilon INC (Shanghai,
83 China). The chemical structures of these analytes are shown in Fig. 1.

84 Water was supplied by J.T.Baker (Gliwice, Poland); acetonitrile, methanol, dimethylsulfoxide
85 (DMSO) and formic acid (98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).
86 All these reagents were LC-MS grade. UTAK Laboratories (Valencia, Spain) was the
87 provider of drug-free heparinized human plasma.

88 2.2. Preparation of calibration standards and internal quality control samples

89 Stock solutions of DAB, OHD and TRA were prepared in DMSO at a concentration of
90 1 mg/mL. Two independent stock solutions were stored separately at -20°C, one for the
91 preparation of calibration standards (CS) and the other for internal quality controls (IQC).
92 Spiking solutions for calibration and control samples were prepared from two sets of working
93 solutions prepared in a mixture of methanol-DMSO (70:30, v/v) at 40 µg/mL and 1 µg/mL for
94 DAB and OHD, at 20 µg/mL and 1 µg/mL for TRA. A stock solution of internal standard (IS)
95 (1 mg/mL) was prepared in DMSO; then diluted in methanol-DMSO (70:30, v/v) to obtain a
96 working solution at 10 µg/mL. All these stock solutions were stored at -20°C in darkness.
97 The day of the analysis, CS and IQC were prepared by diluting spiking solutions with blank
98 plasma. CS consist of six levels: 10, 20, 50, 100, 500 and 2000 ng/mL for DAB and OHD; 5,
99 7.5, 10, 20, 30 and 50 ng/mL for TRA. IQC samples were prepared for low, medium and high
100 concentrations: 25, 200, 1500 ng/mL for DAB and OHD, and 8, 15 and 40 ng/mL for TRA.

101 2.3. Sample preparation

102 A 200 µL aliquot of plasma (CS, IQC and patient sample) was mixed with 20 µL of
103 working solution of IS (10 µg/mL), then vortexed for 5 sec. Six hundreds microliters of
104 acetonitrile with 1% (v/v) formic acid were added for protein precipitation. Samples were
105 vortexed again before centrifugation at 13.000g for 10 min at room temperature (RT). The
106 supernatants were transferred into glass tubes to evaporate to dryness at 45°C under a nitrogen
107 stream. The dried extract was recovered with 200 µL of water-methanol (70:30, v/v) with
108 0.1% (v/v) formic acid, then vortexed before the transfer into a 1.5 mL Eppendorf tube for a

109 second centrifugation (13.000g for 10 min, RT). The final extracts were transferred into a
110 polypropylene vials; then 10 µL were injected into the LC-MS/MS system.

111 2.4. Instrumentation

112 The samples were analyzed using an Accela AS[®] auto-sampler with a quaternary
113 pump and degasser for chromatographic separation (ThermoFisher Scientific, Les Ulis,
114 France). A TSQ QuantumUltra[®] mass spectrometer equipped with an Ion Max electrospray
115 ionization (ESI) interface (ThermoFisher Scientific) was used for mass detection and
116 quantification. Data acquisition and processing were performed using Xcalibur[®] (version
117 2.0.7, ThermoFisher Scientific).

118 Chromatographic separation of three analytes (DAB, OHD and TRA) and IS was achieved on
119 Accucore[®] C18 (2.1 × 50 mm; 2.6 µm) analytical column (ThermoFisher Scientific, Les Ulis,
120 France) associated with a guard column packed with the same bonded phase. Column
121 temperature was maintained at 30°C and the auto-sampler at 4°C.

122 2.5. LC-MS/MS conditions

123 The mobile phase consisted of water (solution A) and acetonitrile (solution B), both
124 with 0.1% (v/v) formic acid. A solution of methanol-water (1:1, v/v) was used to flush the
125 auto-sampler after each injection. The mobile phase was delivered at a flow rate of 500
126 µL/min throughout the 13.2 min-run. The analytes were eluted following this multistep
127 gradient: starting at 95% A and 5% B, the proportion of B is linearly increased to 61.7% up to
128 10.0 min. From 10.0 min to 10.1 min the proportion of B is increased to 90% and the
129 conditions were maintained until 12.1 min. Finally, starting conditions (95% A and 5% B)
130 were maintained from 12.2 min to the end of run at 13.2 min.

131 The chromatography system was coupled with a TSQ Quantum triple quadrupole mass
132 spectrometer (ThermoFisher Scientific, Les Ulis, France). The ESI positive spray voltage was
133 3500 V; the ion transfer tube and vaporizer temperatures were 320°C and 300°C, respectively.

134 The sheath and auxiliary gas were nitrogen at a flow-rate set of 45 and 30 (arbitrary units)
135 respectively; the Q2 (second quadrupole) collision gas (argon) pressure was 1 mTorr. Q1 and
136 Q3, respectively the first and third quadrupole, mass resolution was 0.7 amu full width at half
137 maximum (FWHM). Scan width and scan time were 0.01 amu and 15 msec, respectively.
138 Two optimal m/z transitions were chosen for each analyte: one for quantification and the
139 second one for confirmation in order to have a specific method. Analyses were performed
140 using the selected reaction monitoring (SRM) detection mode. The transitions and general
141 parameters are presented in Table 1.

142 2.6. Method validation

143 The method validation was based on international recommendations [18,19].

144 2.6.1. Limit of quantification and linearity

145 This method was developed for therapeutic drug monitoring and clinical research. The
146 CS values for DAB and TRA were determined from observed concentrations during clinical
147 trials [3,4]. Given the mean OHD-to-DAB exposure ratio in plasma is 0.8 [2], CS values for
148 OHD were similar to those of DAB.

149 Calibration curves for each compound were plotted using the ratio of the analyte's peak area
150 over the IS's peak area versus analyte concentration. The back-calculated concentration for
151 each standard was to be less than 20% of the lower limit of quantification (LLOQ) nominal
152 concentration and less than 15% of the other calibration values.

153 2.6.2. Selectivity and carryover

154 The selectivity of this analytical method was assessed by analyzing plasma samples (n
155 = 10) from polymedicated cancer patients (including BRAF mutated cancer patients) to rule
156 out any interference from others drugs with DAB, OHD, TRA and dabrafenib-d9. The
157 potential interfering signal was to be less than 20 % of the LLOQ for each analyte and less
158 than 5 % for IS. The carry-over was studied by processing blank plasma (n = 6) after the CS

159 at the upper limit of quantification (ULOQ). Carry-over should not be greater than 20% of the
160 LLOQ for each analyte, and 5% of the peak area for internal standard.

161 2.6.3. Accuracy and precision

162 Accuracy was calculated as the percentage of deviation (% bias) between the
163 measured and the nominal concentration. Precision was evaluated as the coefficient of
164 variation (% CV) of the measured concentrations. Intra-day accuracy and precision were
165 assessed by six replicates of LLOQ and each IQC level analyzed in one run the same day.
166 Inter-assay accuracy and precision were evaluated by three replicates of LLOQ and each IQC
167 level on six different days. The analytical assay is considered valid if bias and precision are
168 within $\pm 15\%$ ($\pm 20\%$ for LLOQ and the low IQC).

169 2.6.4. Matrix effect, extraction recovery and process efficiency

170 Three sets of each IQC level (n = 6) with IS were prepared to study matrix effect
171 (ME), extraction recovery (ER) and process efficiency (PE): A) IQC and IS solution were
172 prepared in the recovery phase (water-methanol (70:30, v/v) with 0.1% (v/v) formic acid), B)
173 Blank plasma from 6 different pools were extracted and then spiked with the IQC and IS
174 solution, C) IQC were prepared in 6 different batch of drug-free plasma (UTAK) and
175 processed accordingly to the method described in 2.3.

176 ME, ER and PE were calculated as the ratio of each compounds peak areas (%) of B/A, C/B
177 and C/A, respectively.

178 2.6.5. Dilution integrity

179 Dilution integrity was investigated by charging a plasma sample with the three
180 analytes at a concentration 1.5 times higher than their respective ULOQ, and then by diluting
181 it 2- and 5-fold with drug-free plasma. Dilutions were repeated 6 times. To meet the
182 validation criterion, accuracy and precision were to be within $\pm 15\%$ of the expected value for
183 each dilution.

184 2.6.6. Stability

185 The stability of DAB, OHD and TRA in plasma was investigated in different
186 conditions for each level of IQC (n = 3). Three freeze-thaw cycles were performed with
187 thawing at RT and freezing at -20°C in one day, plasma samples were assayed after storage
188 for 24 hours at RT, 48 hours at 4°C, 30 days and 6 months at -20°C. The stability in the auto-
189 sampler was studied by re-analyzing three levels of IQC (n = 3 for each level) after 48 hours
190 at 4°C. The results were accepted if the mean percentage change from nominal concentration
191 was less than 85%.

192 2.7. Clinical applications

193 The present LC-MS/MS method was used for PK/PD study conducted in mutated
194 BRAF cancer patients treated with DAB and TRA [6]. This study was approved by the local
195 ethics committee in oncology (N° CLEC 211218ACBB1). Patients gave their informed
196 consent before blood collection. In the present study, all patients were concomitantly treated
197 with the recommended daily doses of DAB (150 mg twice a day) and TRA (2 mg once a day).
198 Twenty-five blood samples were collected at steady state in heparin lithium-containing tubes
199 at different time-after-dose over administration intervals. After centrifugation (1850g, 4°C, 10
200 min), until plasma was collected and transferred into polypropylene test tubes before storage
201 at -20°C up to subsequent analysis.

202 Reanalysis of 12 incurred samples (including high and low concentrations) was performed to
203 ensure the reliability of the present method. The two analyses were considered equivalent if at
204 least 67% of the results percentage difference $[(\text{repeat-original})/\text{mean} \times 100]$ was within 20%
205 [19].

206 **3. Results and discussion**

207 3.1. Development

208 Finding the best calibration range was challenging since DAB plasma concentrations
209 are 50 to 100 times higher than TRA concentrations. We based LLOQ and ULOQ on
210 previously published methods and on our experience of TDM [10,12]. Since these molecules
211 are highly lipophilic [3,4], stock solutions were made out of DMSO and the four working
212 solutions in methanol-DMSO (70:30, v/v). Dabrafenib-d9 was chosen as sole internal
213 standard for the three analytes in order to have a simple method that achieved sufficient
214 sensibility.

215 The extraction process and choice of an Accucore[®] C18 column was based on a previously
216 developed method by our laboratory[20] which resulted in an adequate chromatographic
217 separation and satisfactory results. The retention times (RT) and MS/MS settings are reported
218 in Table 1.

219 3.2. Chromatograms

220 Fig. 2 displays typical chromatographic profiles of blank plasma and plasma samples
221 at the LLOQ and ULOQ for the three analytes and the IS.

222 3.3. Limit of quantification and linearity

223 Calibration curves ranged from 10 to 2000 ng/mL for DAB and OHD and from 5 to 50
224 ng/mL for TRA. They were best described by a 1/x weighted linear regression of the ratio of
225 each molecule over IS peak's area versus the concentrations of the respective molecule in
226 each standard sample. Out of 6 calibrations curves, each CS of the three analytes had a CV
227 lower than 8%. Moreover, regression coefficients (r^2) of calibration curves for DAB, OHD
228 and TRA were higher than 0.99 showing a good linearity of the method for each analyte.

229 3.4. Selectivity and carry-over

230 No interference was detected in 10 plasma samples from polymedicated cancer
231 patients for whom we knew their medications. Overall, these plasma samples contained 60

232 different drugs frequently administered in cancer patients. Endogenous signals were lower
233 than 1% of the signal at the LLOQ of each analyte and IS.

234 Regarding carry-over, the signal of blank plasma samples processed after injection of ULOQ
235 samples was less than 5% of the corresponding peak area of the LLOQ sample and the
236 response of the IS was less than 1% of the normal response.

237 3.5. Accuracy and precision

238 The results of accuracy and precision are presented in Table 2. Mean intra- and inter-
239 day accuracies were below 9.3% and 10.9% for each analyte, respectively. Regarding intra-
240 and inter-day precisions, CVs were less than 13.5% and 14.9% respectively. Overall, the
241 results for precision and accuracy met the validation criterion, which proves both the
242 reproducibility and reliability of the analytical method.

243 3.6. Matrix effect, extraction recovery and process efficiency

244 The mean ME, ER and PE ranged from 88.0% to 112.2%, from 61.4% to 87.1% and
245 from 59.1% to 89.9%, respectively (Table 3). The higher polarity of OHD could contribute to
246 its greater ER and PE compared with DAB.

247 3.7. Dilution integrity

248 Accuracy and precision for the 2-fold dilution ranged from -2.1% to 0.2% and from
249 3.1% to 4.3%, respectively; for the 5-fold dilution, from -0.8% to 2.2% and from 3.3% to
250 13.0%, respectively.

251 3.8. Stability

252 Following three freeze-thaw cycles, results met the validation criterion for all analytes
253 (supplementary Table 1). Short-term stability was confirmed at RT and at 4°C, which will
254 allow sufficient time to transport samples from clinical department to the laboratory in daily
255 practice. Long-term stability studies showed that DAB, OHD and TRA were stable at -20°C
256 for up to 6 months. Finally, all analytes were stable up to 48 hours in the auto-sampler at 4°C.

257 3.9. Application to biological samples

258 This new LC-MS/MS method was successfully applied to 25 plasma samples from
259 BRAF-mutated cancer patients including metastatic melanoma (n = 20), NSCLC (n = 2) and
260 ATC (n = 3). The median concentrations [range] were 332 ng/mL [36-2273], 335 ng/mL [29-
261 1416] and 12.6 ng/mL [5.5-21.6] for DAB, OHD and TRA respectively. As previously
262 reported, trough and peak concentrations for DAB and TRA were in the expected range for
263 patients treated with the recommended daily dose. In the same way, median OHD-to-DAB
264 ratio was 0.8 [0.5-2.8], which is in accordance with the expected value [2]. The results of the
265 incurred sample reanalysis showed the lack of any significant difference (<20%) between the
266 initial analysis and the second one for all the analytes (Fig. 3), which confirmed the satisfying
267 reproducibility observed with IQC. Taken together, these results prove the suitability of our
268 method to monitor DAB, OHD and TRA in plasma from BRAF-mutated cancer patients.
269 Vikingsson et al. [17] reported a method to assay DAB and six metabolites but only through
270 semi-quantitative calculation using the calibration curve for DAB. To our best knowledge, the
271 present method is the first to simultaneously quantify DAB and its main active metabolite
272 OHD. We recently reported that plasma ratio $AUC_{OHD}/AUC_{DAB} \geq 1$ is independently
273 associated with shorter overall survival (Hazard Ratio: 10.61 (2.34-48.15), $p < 0.05$), which
274 suggests a clinical benefit to monitor OHD in daily clinical practice [6].
275 Finally, this method offers the advantage of simultaneously monitoring TRA in patients
276 treated in association with DAB and TRA. This may contribute to more widespread plasma
277 monitoring of these drugs in BRAF-mutated cancer patients in daily clinical practice.

278 4. Conclusion

279 We have developed and validated a LC-MS/MS method to simultaneously quantify
280 DAB, OHD and TRA in human plasma. Patients treated with DAB and TRA exhibit a large
281 interindividual variability in clinical outcomes in terms of toxicity and efficacy, which has led

282 to a growing interest in PK/PD investigations. This method is suitable for studying OHD
283 pharmacological activity especially in daily clinical practice.

284 **CRedit authorship contribution statement**

285 **David Balakirouchenane:** Data curation, Formal analysis, Methodology,
286 Visualization, Writing - original draft. **Nihel Khoudour:** Formal analysis, Methodology,
287 Supervision, Writing - review & editing. **Sarah Guégan:** Data curation, Investigation,
288 Writing - review & editing. **Nora Kramkimel:** Data curation, Investigation, Writing - review
289 & editing. **Nathalie Franck:** Data curation, Investigation, Writing - review & editing.
290 **Thomas Rodier:** Data curation, Writing - review & editing. **François Goldwasser:** Funding
291 acquisition, Writing - review & editing. **Nicolas Dupin:** Data curation, Investigation, Writing
292 - review & editing. **Selim Aractingi:** Data curation, Investigation, Writing - review & editing.
293 **Michel Vidal:** Conceptualization, Methodology, Resources, Supervision, Writing - review &
294 editing. **Benoit Blanchet:** Conceptualization, Methodology, Supervision, Writing - review &
295 editing.

296 **Declaration of Competing Interest**

297 Benoit Blanchet has served on advisory boards and received honoraria from
298 AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Leo Pharma, and Pfizer. Francois
299 Goldwasser has received a travel, accommodation, and research grant from Bristol-Myers
300 Squibb. Nicolas Dupin reports personal fees, travel, accommodations, and expenses from
301 Roche. Selim Aractingi has been a speaker for Pierre Fabre, Novartis, Janssen, and Abbvie
302 and a member of boards of meetings for BMS and Sanofi.

303 Other authors have declared no conflict of interest.

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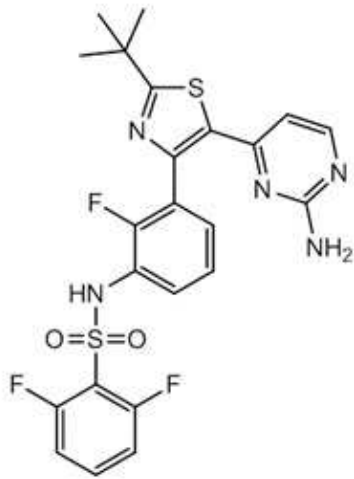
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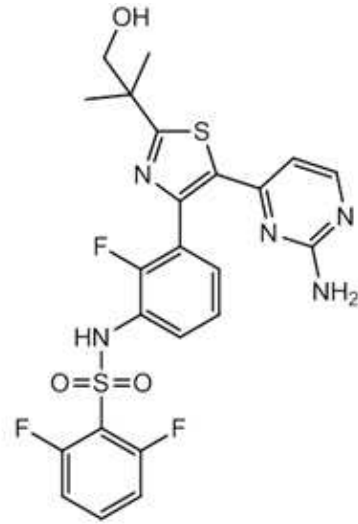
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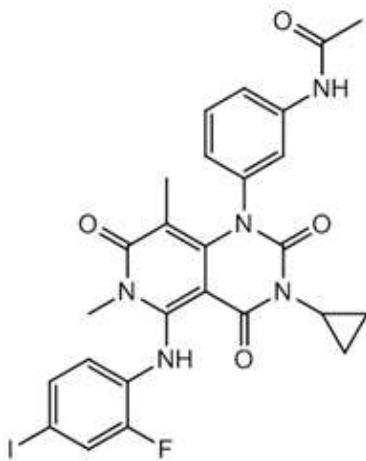
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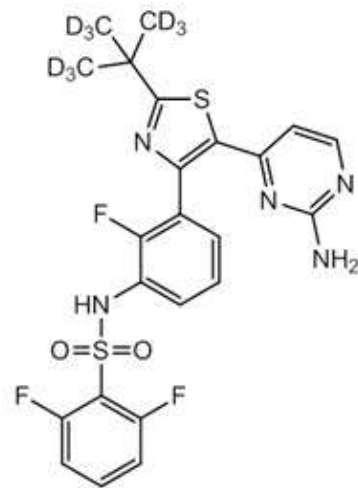
Dabrafenib



Hydroxy-dabrafenib



Trametinib



Dabrafenib-d₉

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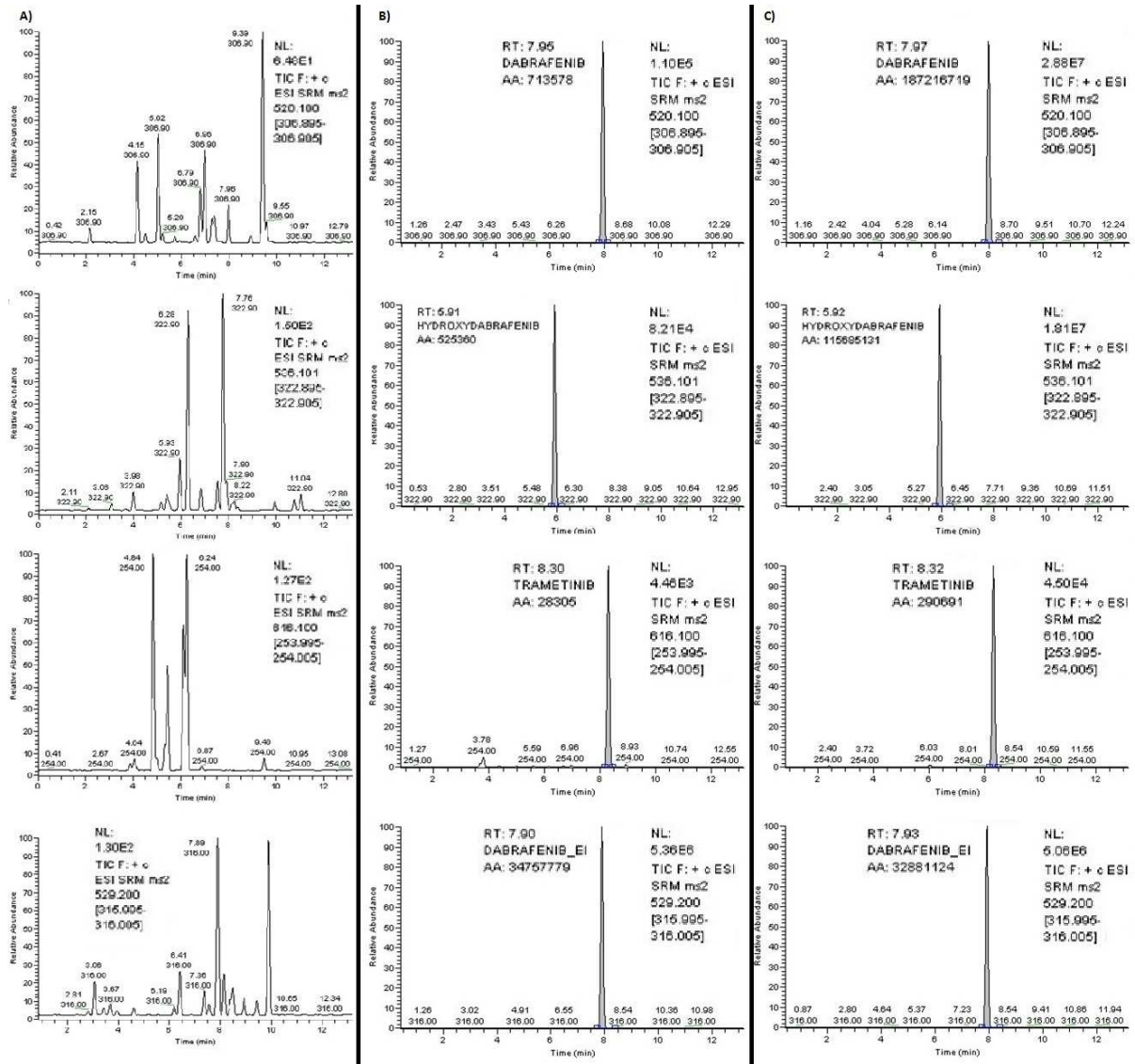
Fig 1. Chemical structures of the analyzed drugs, metabolite and internal standard.

396 **Table 1**
 397 MS/MS general settings for dabrafenib (DAB), hydroxy-dabrafenib (OHD), trametinib (TRA)
 398 and the internal standard (IS).

Analyte	Expected RT (min)	ESI	Precursor ion (m/z)		Product ion (m/z)	CE (eV)	TL (V)
DAB	7.9	+	520.1	Quantification	306.9	29	154
				Confirmation	276.9	57	
OHD	5.9	+	536.1	Quantification	322.9	30	121
				Confirmation	292.9	44	
TRA	8.2	+	616.1	Quantification	254.0	37	124
				Confirmation	491.0	30	
IS	7.8	+	529.2	Quantification	316.0	30	142
				Confirmation	279.9	66	

399 CE, Collision Energy; eV, electron volt; IS, internal standard; ESI, Electrospray Ionization; m/z, masse/charge; min, minute; RT, Retention Time; TL, Tube Lens; V, volt.

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AA, abundance area; NL, noise level; RT, retention time; SRM, selective reaction monitoring; TIC, total ion current.

Fig. 2. Chromatograms of A) blank plasma, B) the lower limit of quantification and C) the upper limit of quantification containing dabrafenib, hydroxy-dabrafenib, trametinib and internal standard, from top to bottom respectively.

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457 **Table 2**
 458 Intra- and inter- assay accuracy and precision of dabrafenib (DAB), hydroxy-dabrafenib
 459 (OHD) and trametinib (TRA) at lower limit of quantification, low, medium and high
 460 concentrations.

Nominal concentration (ng/mL)	Level	Intra-day accuracy (% bias, n = 6)	Intra-day precision (% CV, n = 6)	Inter-day accuracy (% bias, n = 18)	Inter-day precision (% CV, n = 18)
DAB					
10	LLOQ	8.1	4.3	9.9	4.0
25	IQC1	1.3	2.0	4.2	6.9
200	IQC2	6.9	2.3	1.6	8.6
1500	IQC3	0.9	3.1	10.9	4.8
OHD					
10	LLOQ	5.2	6.7	6.2	7.8
25	IQC1	0.9	4.1	1.2	6.6
200	IQC2	2.9	3.3	-1.2	4.7
1500	IQC3	5.9	4.8	10.1	2.8
TRA					
5	LLOQ	6.6	12.3	0.6	11.0
8	IQC1	1.4	12.5	2.5	14.9
15	IQC2	3.9	10.5	2.5	10.4
40	IQC3	9.3	13.5	0.8	8.7

CV, Coefficient of variation; IQC, internal quality control; IQC1, IQC2 and IQC3 correspond to the IQC concentrations at low, medium and high level, respectively; LLOQ, lower limit of quantification

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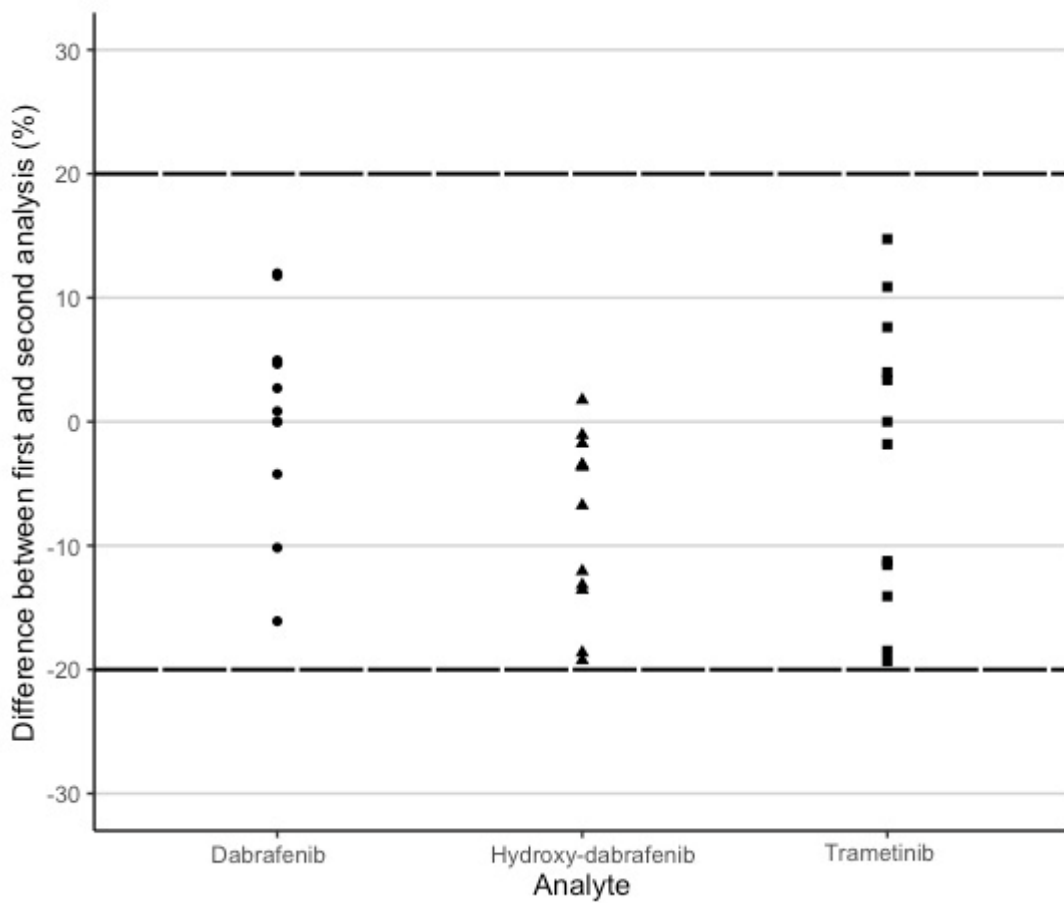


Fig. 3. Percentage difference of the samples reanalysis for dabrafenib, hydroxy-dabrafenib and trametinib (n = 12 for each analyte).

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516 **Table 3**
 517 Recovery and matrix effect for dabrafenib (DAB), hydroxy-dabrafenib (OHD), trametinib
 518 (TRA) and internal standard (IS) in human heparinized plasma samples (n = 6 for each
 519 analyte and n = 18 for the IS).

Nominal concentration (ng/mL)	Level	Matrice effect (%)		Extraction recovery (%)		Process efficiency (%)	
		Mean	CV	Mean	CV	Mean	CV
DAB							
25	IQC1	94.6	5.7	64.2	7.6	60.7	9.0
200	IQC2	96.5	2.2	65.2	9.5	62.9	9.2
1500	IQC3	88.0	6.5	67.4	10.4	59.1	10.0
OHD							
25	IQC1	104.5	6.1	85.3	5.0	86.7	10.6
200	IQC2	106.9	3.6	86.3	4.1	89.9	4.5
1500	IQC3	93.7	4.5	87.1	4.3	81.5	2.9
TRA							
8	IQC1	112.2	13.8	61.4	6.1	68.6	12.9
15	IQC2	105.1	13.8	67.6	10.7	67.6	14.7
40	IQC3	99.8	11.8	66.3	11.8	63.2	5.4
IS							
		93.9	5.3	70.1	9.0	65.7	9.7

520 CV, coefficient of variation. IS, internal standard.